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La Revue *Proceedings of the Nova Scotian Institute of Science* publie les travaux de l'Institut, des articles originaux et des notes avant trait aux sciences naturelles aux Provinces atlantiques, surtout dans les domaines de la biologie et de la géologie. Les manuscrits peuvent être en anglais ou en français, et seront soumis à l'avis d'experts avant d'être acceptés.

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Les tableaux taxonomiques doivent se présenter, dans des cas de la zoologie et de la paléontologie, sous forme de descriptions dichotomiques alignées verticalement, et pour la botanique sous forme multilinéaire étagée. La synonymie, en botanique, en zoologie et en paléontologie, doit être représentée succinctement: taxon, auteur, année, page, avec citation au complet aux indications bibliographiques.

Un résumé ne dépassant pas 200 mots doit précéder le corps du texte. Le texte lui-mème doit comprendre les sections suivantes: introduction, méthodes, résultats ou observations, discussion, références bibliographiques. Dans certains cas, une systhèse de deux ou de plusieurs de ces sections peut étre souhaitable.

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A SURVEY OF INFESTATION OF CROPS BY STRIGA SPP. IN BENIN, NIGERIA AND TOGO

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The incidence of *Striga* spp. on agricultural crops in Benin, Nigeria and Togo between latitudes 4° N and 13° 30′ N was surveyed in 1984 and 1985. The survey covered 463 fields in Nigeria, 112 in Benin and 55 in Togo. It was found that the *Striga* spp. responsible for significant commercial losses were *S. hermonthica, S. asiatica,* and *S. aspera.* In Nigeria *S. hermonthica* and *S. aspera* were frequently responsible for 100% loss of the crop. In the maize growing areas of Southern Benin and Togo the crop was mainly infested with *S. asiatica. S. aspera* is recorded on lowland *Oryza sativa* for the first time. Evidence is presented for the growth of *S. hermonthica* on *Arachis hypogea.*

Au Benin, au Nigeria et au Togo la présence du Striga, mauvaise herbe parasitaire se situe entre latitude 4° N et 13° 30′ N. En 1984 et 1985 une prospection de 463 champs au Nigeria, 112 au Benin et 55 au Togo a montre que les espèces de parasite provoquant les dégâts significatifs sont les *S. hermonthica, S. asiatica* et *S. aspera.* Les perdes de récolte s'élevant fréquemment au 100% au Nigeria sont dues a *S. hermonthica* et *S. aspera.* Dans les champs de mais au sud du Benin et Togo l'espèce les pluis importantes sont les *S. asiatica.* Pour la premiere fois, on observe l'infestation du riz irrigué par une espèce de Striga, le *S. aspera.* On note également pour la premier fois que l'arachide (*Arachis hypogea*) est attaquée par *S. hermonthica.*

Introduction

Striga species are root parasitic weeds that cause major losses to cereal crops in Africa. The biology and taxonomy of the species have been reviewed (Musselman, 1980). The parasites causes extensive losses on maize (*Zea mays*) through growth reduction, poor pollen production, barrenness, weakening of the stem and mortality. They are equally harmful on other cereal crops such as sorghum (*Sorghum vulgare*), millet (*Pennisetum glaucum*), rice (*Oryza sativa*), hungry rice (*Digitaria exilis*) and sugar cane (*Saccharum officinarum*). *Striga gesneroides* is parasitic on *Vigna unguiculata* (cowpea). Maize is one of the most important cereal crops in Western and Eastern Africa and is replacing sorghum in areas where the rainfall is greater than 600 mm y^{-1}. In the savanna of Northern Nigeria, maize production has increased by 46% in the period 1980 to 1985 (or from 7% to 10.6% of the total cereal crop; United Nations, 1985). In the South, maize farming has also increased by about 40% in the derived savanna zone of the rainforest (Fajamisin, 1985). In Benin and Togo where maize is relatively more important (80% of the cereal crop in Benin and 48% in Togo) production has also increased by 24% and 36% in the period 1980 to 1985 (United Nations, 1985).

Maize, however, is a relatively new crop in the sorghum and millet growing areas and it is more vulnerable to infestation by *Striga* spp. but the distribution and relative importance of the weed is not well documented. Thus the object of this survey was to determine the distribution and severity of *Striga* spp. on maize in particular and other crops in general. Hopefully this will enable the Maize Improvement Program at the International Institute of Tropical Agriculture implement its mandate which is centered on the development of high yielding, pest and disease resistant varieties for Africa.

Methods

Surveys were conducted over a two year period (1984 & 1985) from June to October in each year, with at least 2 visits to each area (Cochran, 1977). This was necessary because planting dates varied and even though symptoms of *Striga* infestation were evident on the crop, it was important for identification purposes to visit when the parasite was in flower.

The area surveyed extended from latitude 4° 30′ N to 13° 30′ N in Nigeria; 5° 30′ N to 12° N in Benin and 6° N to 11° N in Togo. Villages to be visited were selected at random before the trip was undertaken. Seventeen out of 19 States in Nigeria were surveyed, at least 10 farms were visited in each administrative zone. In Benin and Togo, the survey was conducted in all provinces. Administrative zones in these countries are much smaller than in Nigeria and therefore, all farms were visited in

Table I Distribution (%) of *Striga* on *Zea mays*.

States	No. of fields sampled	*S. hermonthica*	*S. aspera*	*S. asiatica*	Other Spp.
NIGERIA					
Anambra	27	0	0	19	0
Bauchi	34	12	68	0	0
Bendel	10	0	0	0	0
Benue	43	40	74	23	0
Borno	24	29	25	0	29*
Cross River	10	0	0	0	0
Gongola	43	21	61	0	0
Imo	10	0	0	0	0
Kaduna	39	64	31	0	5′
Kano	19	100	0	0	5′
Kwara	49	33	45	22	8″
Niger	57	86	19	11	0
Ondo	10	0	0	0	0
Oyo	20	0	0	25	0
Plateau	39	69	65	15	5+
Rivers	10	0	0	0	0
Sokoto	19	58	0	0	0
BENIN REPUBLIC					
Atakora	30	57	23	47	
Atlantique	5	0	0	0	
Borgou	34	59	15	50	
Mono	10	0	0	50	
Oueme	6	0	0	0	
Zou	27	0	41	48	
TOGO					
Centrale	10	30	50	70	
Maritime	12	0	0	83	
Plateau	9	0	0	78	
Kara & Savannes	24	88	46	38	

* *Striga parssargei;* ′ *S. brachycalyx;* ″ *S. forbesii;* + *Buchnera hispida.*

areas with less than 10 maize farmers. On farms of less than 10 ha, the entire field was surveyed; on those over 10 ha, 10 plots with a basal area of 10 m^2 were established at intervals of 50 m, starting from the corners and working towards the center of the field. Interviews with farmers were always conducted at the farm and occasionally farmers in small villages requested a group discussion.

Surveys of the weed and its hosts were based on the presence or absence of the parasite. A field was considered infested if at least one *Striga* plant was found. Visual estimation of the percentage of crop plants infested was made and rated as follows: 0 = no visible sign of infection; 1 = less than one-third of the field affected; 2 = two-thirds infected; 3 = more than 2/3 infected. The *Striga* population was also visually estimated and was rated 1 for low; 2 = moderate and 3 = heavy infestation. In Table II these ratings were given under the headings "Effect on crop" and "Parasite density" respectively.

Results

NIGERIA

Infestation of Zea mays by Striga spp. In a country with a diversified ecology ranging from the rain forest to the arid sahel, *Striga* was present in varying degrees from slight to heavy infestation in all but the high rainforest zone (Table I). A total of 6 species were found on maize, 3 of which were of economic importance.

Striga hermonthica is characterized by large bright pink flowers with a five-ribbed calyx tube. The plant is erect, highly branched and can reach a height of 80 cm. It occurred in 10 out of 12 States where the weed was present (Fig 1, Table I) and was predominant in the drier northern-most zones causing serious loss in yield in the following States: Niger, Kaduna, Kano, Sokoto, Plateau and upper Benue. All the farms with a heavy *Striga* rating (3) in these areas were abandoned (Table II).

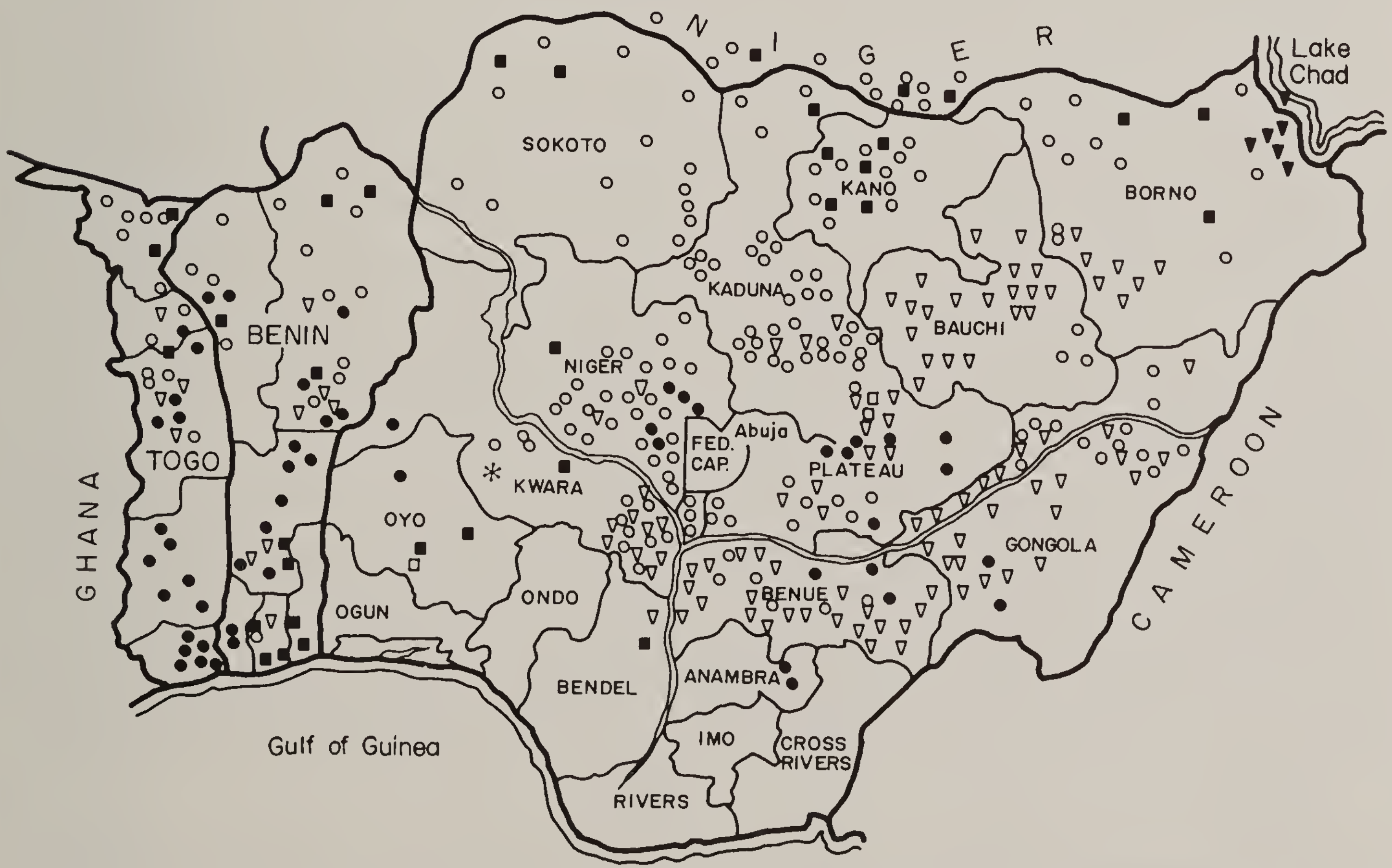

Fig 1 Map of area surveyed. Distribution of species indicated as follows: o = *Striga hermonthica*; ● = *S. asiatica*; △ = *S. aspera*; ■ = *S. gesneroides*; □ = *S. forbesii*; * = *S. brachycalyx*; ▲ = *S. passargei*.

Striga aspera is similar to *Striga hermonthica* except that the width of the corolla is smaller; the corolla tube is 0.5-1 times longer than the five ribbed calyx tube, and the seed capsules are bigger. The plant is less branched with much narrower leaves and less foliage. Usually a high proportion of plants have purple pigmentation throughout the entire plant. This species was more important in the wetter areas of the Guinea and woodland savannahs where the mean annual precipitation is greater than 100cm. *Striga aspera* appeared to be virulent on maize resulting in stunting and death of the crop before tasseling. Many farms with moderate to heavy infestation were abandoned in the Guinea savannah zone of Bauchi, lower Benue, upper Plateau and in Kwara States (Table II). Niger State was an exception where *Striga hermonthica* was predominant in a woodland savannah ecology.

Striga asiatica is a much smaller plant which grows up to a height of about 30 cm. The flowers are small and the corolla measured 5-10cm in diameter with a ten ribbed

Table II Mean *Striga hermonthica, S. aspera* and *S. asiatica* ratings for densities of parasites and effects on crops in the States of Nigeria, Benin and Togo.

States	*S. hermonthica* Parasite density	*S. hermonthica* Effect on crop	*S. aspera* Parasite density	*S. aspera* Effect on crop	*S. asiatica* Parasite density	*S. asiatica* Effect on crop
NIGERIA						
Anambra	0	0	0	0	1	1
Bauchi	2	2	3	3	0	0
Bendel	0	0	0	0	0	0
Benue	2	3	3	2	2	2
Borno	3	3	2	2	0	0
Cross River	0	0	0	0	0	0
Gongola	3	2	3	2	1	1
Imo	0	0	0	0	0	0
Kaduna	3	2	1	2	0	0
Kano	3	2	0	0	0	0
Kawara	3	2	3	3	2	2
Niger	3	3	3	3	2	2
Ondo	0	0	0	0	0	0
Oyo	0	0	0	0	1	1
Plateau	3	3	2	3	2	2
Rivers	0	0	0	0	0	0
Sokoto	3	2	0	0	0	0
BENIN REPUBLIC						
Atakora	3	3	2	2	2	2
Atlantique	0	0	0	0	0	0
Borgou	3	3	2	2	2	2
Mono	0	0	0	0	2	3
Oueme	0	0	0	0	0	0
Zou	0	0	2	2	1	1
TOGO						
Centrale	2	2	2	2	2	2
Maritime	0	0	0	0	3	3
Plateau	0	0	0	0	2	1
Kara & Savannes	3	2	2	2	2	2

calyx tube. The yellow flowered variety was the only species found in a few farms in Anambra and Oyo States. It was also present in Kwara State where, in some cases, it was the only species on maize. The occurrence of this morphotype was the only record of *Striga* in the rain forest ecology at Erin Ile at latitude 8° 1′ N, northeast of Ibadan on the border of Kwara and Oyo States. In Niger and Benue States, it was usually in association with *Striga aspera* and *Striga hermonthica*. The red flowered variant occurred in Plateau State and the white flowered morphotype was found in Gongola State; its effects on the crops were less serious at both locations.

The intensity and severity of these 3 species across the country ranged from 50-100% of all fields sampled (Table II), except in Anambra and Oyo States.

Striga brachycalyx was present mostly in the northern parts of Kwara State and caused slight damage to maize on the few farms where it occurred. The plants attained a height of about 30 cm, sparsely branched with leaves much more narrowly lanceolate than those of *Striga aspera*; flowers were light pink with a five ribbed calyx tube.

Striga passargei was found on maize growing in the bed of Lake Chad in Borno State. The plant was highly branched and grew to a height of *ca.* 30 cm with lanceolate to broad leaves, white flowers with a corolla diameter of 7 mm and a five ribbed calyx tube. Its severity on the crop was low to moderate. It was also present in a few farms in Kwara State.

Buchnera hispida a close relative of *Striga*, with blue flowers, occurred in Kaduna and Kano States. The sparsely branched plant grew to a height of 30-80 cm, five petals measured 4-7 mm in diameter with a ten ribbed calyx tube. Although it was found parasitizing maize, sorghum and millet, it was not of economic significance.

Infestation of other Agricultural Crops by Striga spp. Sorghum and millet were seriously attacked in the Sahel and Sudan savannah zones by *Striga hermonthica* which was the predominant species in areas whose annual rainfall was less than 80 cm. These crops were also seriously attacked by this species and by *S. aspera*, and *S. asiatica* in Bauchi, Niger and Gongola States. In general sorghum was more infested than millet.

Upland rice was infested by *Striga hermonthica* in Gongola State whereas lowland rice was heavily infested by *S. aspera* in this State and in Plateau State, reducing the potential yield by over 70% in some cases. These observations were made at a few rice farms during the survey and do not indicate the effect of the weed on the rice crop in general. *Digitaria exilis* (hungry rice), a small grain crop was attacked by *S. aspera* and the red flowered variety of *S. asiatica* in Plateau State. Sesame (*Sesamum indicum*) was infested by *S. asiatica* in Benue and by *S. gesneroides* in Kano State.

Groundnut crops (*Arachis hypogea*) in Bauchi State were heavily parasitized by *S. aspera* and moderately infested by *S. hermonthica* in Niger State (cf. Andrews, 1947). Almost all incidence of *Striga* on this crop were on farms where maize or sorghum was intercropped with it and the cereal crop had been killed by the parasite. One incidence of infestation was observed on groundnuts where this was the only crop, but no apparent damage to the host was seen (Fig 2). Infestation of cowpea by *S. gesneroides* occurred only in the Sudano-sahelian savannah region where the mean annual rainfall was $<$ 80 cm. The pink flowered variant of this species occurred in Niger State but not on the crop — it was found as a parasite on the wild legume *Arachis repens*.

BENIN REPUBLIC

Incidence of Striga spp. on Zea mays. Striga spp. were present in all Provinces. However, there was little incidence of *Striga* on maize in the southern provinces of Oueme and Atlantique; farmers reported sporadic infestation in some years.

Striga hermonthica was predominant in the two northern provinces of Atakora and Borgou and was practically the only species further north near the borders with Niger and Burkina Faso (Fig 1, Table I).

Striga aspera was more serious in the Central Province of Zou where many farms were abandoned. The crop in an entire village in the Djonta Kpassagon region was severely infested.

Striga asiatica: the yellow flowered variant was commonplace in Northern Zou and Southern Borgou Provinces. It also occurred in the North-western part of Atakora but was of lesser importance than *S. hermonthica*. The red flowered variant was present in the province of Mono and the white flowered variant was found on a few farms in Atakora.

Infestation of other crops by Striga spp. Other crop hosts were sorghum and millet in the northern parts of Atakora and Borgou Provinces where they were heavily infested by *S. hermonthica*. Cowpea served as the host of *S. gesneroides* in the provinces of Oueme, Atlantique and Zou. Farmers claimed that most of the cowpea consumed in Benin used to be produced in Zou Province but in the last 5 years, this is no longer true because of *Striga* infestation.

TOGO

In Togo *Striga* spp. were present in all regions of the country. In the savanna regions of the north 87.5% of the maize farms visited were infested with *Striga hermonthica*. It was also present in the central region of the country but was not the most important species, where *S. aspera* was most important (Table I). The latter species also occurred frequently in the savanna region.

S. asiatica the red flowered variant on maize was only observed in the maritime region. It occurred in 83% of all the fields sampled and caused such severe damage to the crop that fields were abandoned. Beyond this region, the yellow flowered variant was the representative of the species generally found. Sorghum and millet were the other food crops attacked by this species in northern Togo. No incidence of infestation of cowpea was found but the pink and white flowered *S. gesneroides* were seen on *Arachis repens.*

Discussion

The survey showed that the most widely distributed species was *Striga hermonthica* (Fig 1), found ubiquitously on sorghum and millet in the Sahel. There is evidence (King and Zummo, 1977) for physiological specialization of this species and these crops. *S. hermonthica* is widely distributed in Africa (Doggett, 1965; Musselman, 1980; Musselman and Riley, 1984; Parker, 1984) and even at low infestation rates, dwarfing of the crop suggests toxic systemic effects. *S. aspera* appeared to be more virulent on maize, also causing damage at low frequencies of infestation (Table II). This species seemed to be better adapted to areas with higher humidity (Fig 1) epitomized by its infestation of rice in water-logged conditions. *S. asiatica* was at the least important of the 3 major species in Nigeria but was the only species found on maize in southern Togo, and in general appeared to be more common than *S. aspera* in most of that country.

The seed of *Striga* is dispersed by wind and water, particularly by dust storms during the harmattan, but also by the practice of grazing cattle in infested fields and their migration across borders in search of feed. Thus the imposition of quarantine regulations is neither feasible nor practical. The distribution map (Fig 1) suggests that the weed tolerates wide variations in soil type, but during the survey it was noticed that *S. aspera* in Togo was usually found in highly weathered soils with a high gravel component. It is therefore possible that detailed ecological studies might establish

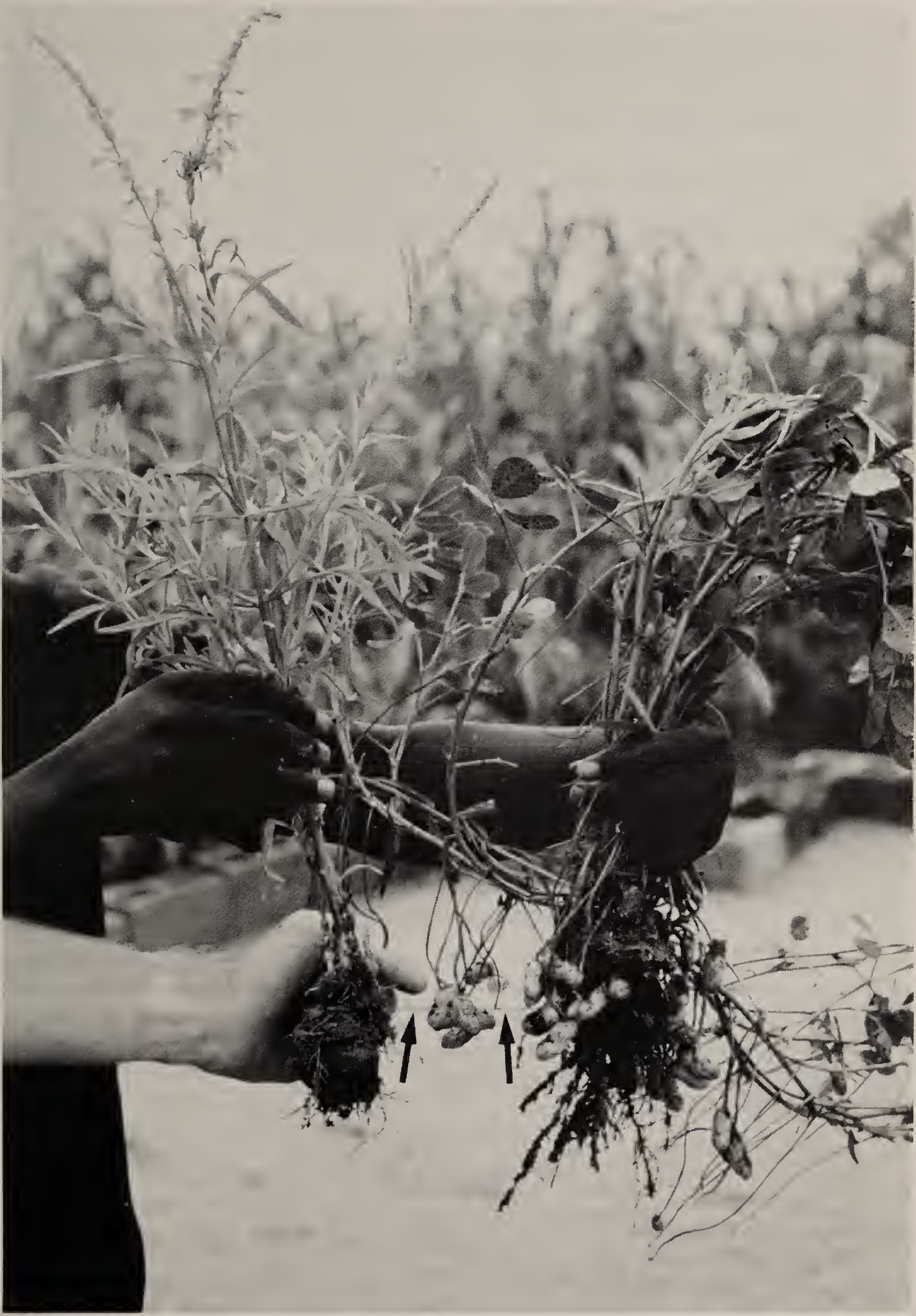

Fig 2 Photograph of roots of *Arachis hypogea* infested with *Striga hermonthica*.

a relationship between the distribution of *S. aspera* and soil type.

Some indication of possible methods of control of *Striga* were observed during the survey. For example, in areas where the mean annual rainfall was > 80 cm millet appeared to be resistant to *S. aspera*. It is possible that the root system of a crop adapted to high soil temperatures and drought does not secrete germination stimulants for *S. aspera*. Cotton is an important crop in Benin and Togo and is known to produce the germination stimulant strigol (Cook, 1972). Cotton is resistant to infestation by the weed but induces its suicidal germination. In practise good yields of maize were often seen when it was grown after cotton, but this effect only persisted for one cropping season. Hand weeding, hoeing and ploughing the soil were also found as attempts to control the growth of *Striga* on farms. Of these methods hand weeding was the most effective because the scale buds on the underground stem were uprooted. Hoeing and ploughing were ineffective because they destroyed only the aerial parts of the plant and, in fact, stimulated the scale buds to grow.

During the survey many discussions with farmers were held. In general, farmers were aware of the scale of the problem and the local names for the weed e.g. killer weed, fireweed, indicate their apprehension as the symptoms on the host made their appearance. Their general practice is to abandon infested fields and there were cases of relocation of families from Atakora to Borgou Province in Benin. Farmers are unaware of the life-cycle of *Striga*. For example bush fallow is often practiced and farmers are surprised that even after 5 years fallow maize becomes infested as they do not know that *Striga* seeds can remain viable in the soil for 20 years (Doggett, 1965). Even large farm operators do not appreciate the consequences of their actions. For example during the survey a large crop (1000 ha) of dwarf millet was examined. It was heavily infested with *S. hermonthica* but appeared to be resistant to the parasite which grew as tall as the crop — which was ultimately harvested by a combine.

The problem of *Striga* infestation is complex and much information needs to be disseminated to farmers, in particular details of agronomic practices designated to curtail the growth of the weed. In the meantime breeding varieties for resistance to *Striga* seems to be the most promising approach to improve crop production.

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ESTIMATES OF THE NUMBERS AND AREAS OF ACIDIC LAKES IN NOVA SCOTIA

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There are 6674 lakes larger than 1 hectare in Nova Scotia covering an area of 2255 km². Geological and pH isopleth maps were consulted to estimate acidified and acid sensitive lakes. Assuming that granitic or metamorphic bedrock only very slowly produce acid neutralizing ions, we estimate that 78% of the lakes (85% of lake area) would, in the absence of moderating influences of surficial geology and marine aerosols, be susceptible to acidification. When all sources of acid neutralizing capacity are indirectly considered via examination of pH isopleths drawn from lake chemistry, we estimate that 16% of the lakes (26% of lake area) have zero alkalinity, and that 69% of the lakes (80% of lake area) have $< 50\ \mu\text{eq L}^{-1}$ alkalinity.

En Nouvelle-Ecosse il y a 6674 lacs ayant une superficie au delà d'un hectare, avec une superficie totale de 2255 km². On a consulté des cartes géologiques ainsi que des cartes d'isoplèthes de pH, en but d'obtenir une estimation des lacs acidifiés ainsi que des lacs sensibles à l'acide. Supposant que le fond de roche granitique ou métamorphique libère des ions qui neutralisent l'acide très lentement, nous estimons que 78% des lacs (85% des superficies) seraient predisposés à l'acidification, dans l'absence d'influences modérantes de la geologie superficielle. Quand on donne leurs poids à toutes les sources démontrant la capacité de neutraliser l'acide, par le biais d'inspection des isoplèthes de pH des lacs, nous estimons que 16% des lacs (26% de la superficie totale) ont nulle alcalinité, et que 68% des lacs (80% de la superficie totale) ont une mesure d'alcalinité de $50\ \mu\text{eq L}^{-1}$.

Introduction

The Dominion Water Power Branch of the Canada Department of the Interior initiated hydrometric surveys in Nova Scotia in 1915 and prepared a drainage index map which divided the 55.5×10^3 km² province into 44 basins (Fig 1). In 1980 a series of 1:50000 Watershed Area maps of the province was compiled by Maritime Resource Management Survey. From these maps the areas of all lakes over 1 hectare (the smallest size for which statistics were available on the maps) were tabulated for each of the 44 drainage areas by B. Sabean, Nova Scotia Department of Lands and Forests (unpubl. data). We have used Sabean's data, geological data (Anon. 1986), and water chemistry information (Clair *et al.* 1982, Alexander *et al.* 1986 and Underwood *et al.* 1987) in order to provide estimates of the numbers and areas of acidic lakes in Nova Scotia.

Nova Scotia lakes are sensitive to acidic precipitation (Kerekes *et al.* 1982; Watt *et al.* 1983; Underwood *et al.* 1987) and while estimates of the proportions of lakes in various classes of acidity have been published (Jeffries *et al.* 1986; Kelso *et al.* 1986; Underwood *et al.* 1986), none have had the advantage of working with information about the actual number of lakes in the province as is the case in this study.

Methods

Lakes were subdivided into pH classes by superimposing a lake pH isopleth map (Underwood *et al.* 1987; Fig 2) and calculating isopleth areas by planimeter. The total number of lakes within one drainage unit was assumed to be evenly distributed throughout the unit.

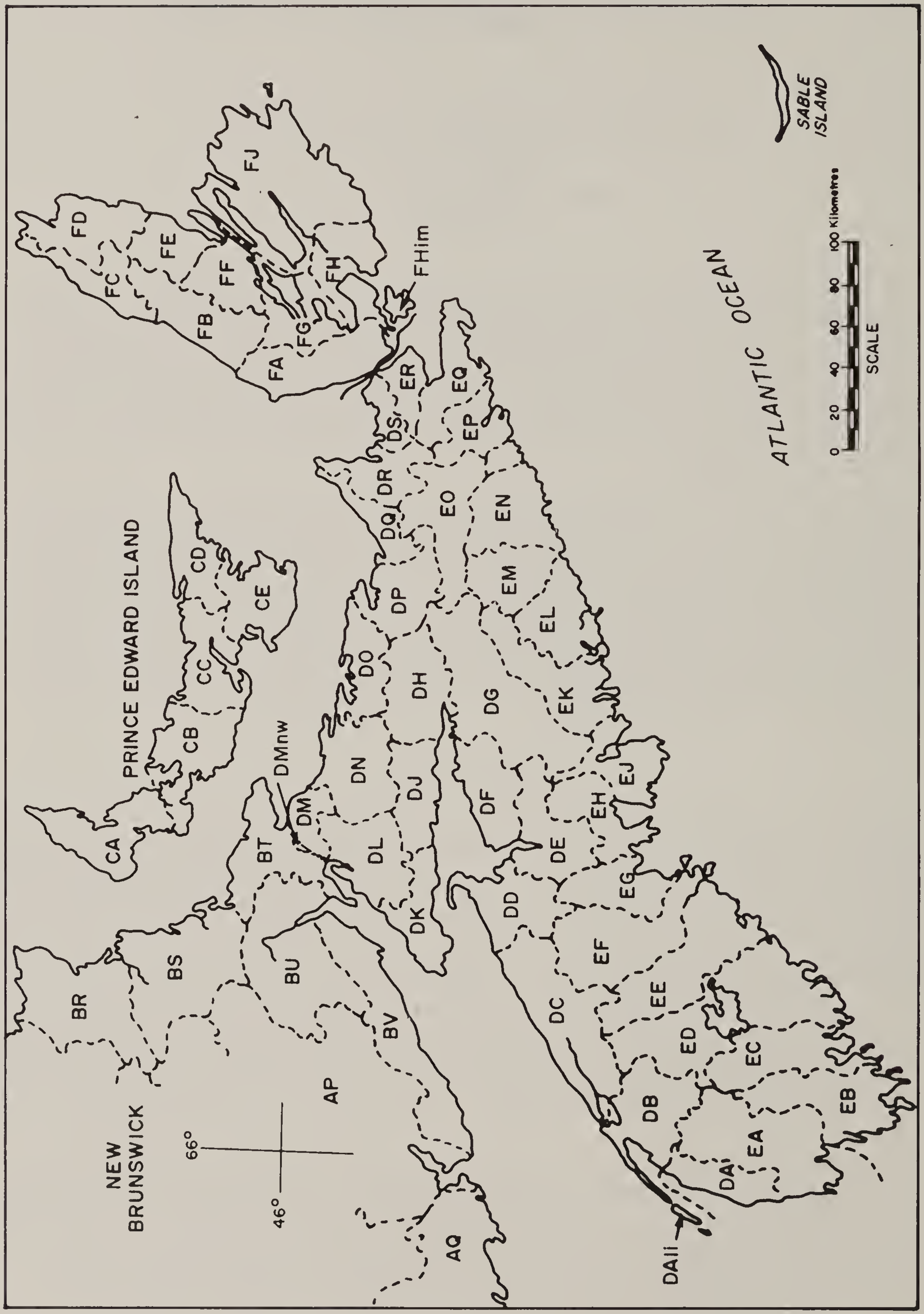

Fig 1 Map of 44 drainage basins in Nova Scotia plus several in New Brunswick and Prince Edward Island. The nomenclature designating each Nova Scotian drainage basin is used in Table II.

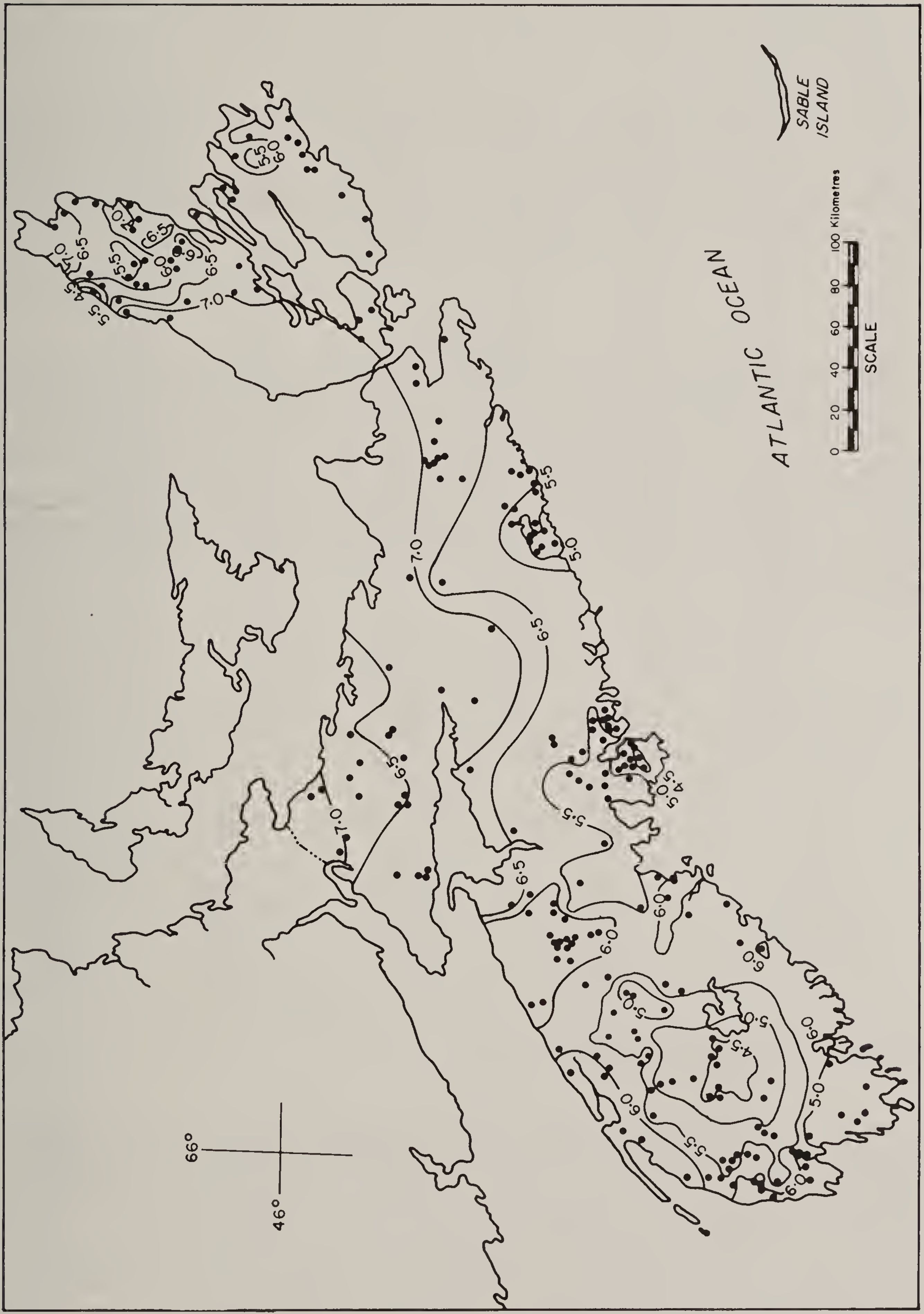

Fig 2 pH Isopleths for lake waters in Nova Scotia.

Kelso *et al.* (1986) classify lakes by both pH and alkalinity with one group for example listed as alkalinity $< 50 \mu eq\ L^{-1}$. Underwood (1984) developed an equation for determination of HCO_3^- from pH measurements in precipitation:

$$HCO_3^- (\mu eq\ L^{-1}) = 11.35/H^+ (\mu eq\ L^{-1}) \quad (1)$$

where $H^+ = 10^6 \cdot 10^{-pH}$

Using this equation we have calculated that $50 \mu eq$ Alk L^{-1} (as HCO_3^-) is equivalent to pH 6.6 which was interpolated on the pH isopleth map of Underwood *et al.* (1987) to provide estimates for lakes with alkalinity $< 50 \mu eq\ L^{-1}$. A value of 6.6 is mid point of lakewater pH as predicted by Wiltshire and Machell (1981) for CO_2 saturated and supersaturated conditions and $50 \mu eq\ L^{-1}$ alkalinity.

Results

Table I shows total numbers and percentages of several categories of lakes with surface areas > 1 hectare. The lake number is lower than estimates of Smith (1963) who reported 2580 lakes over 10 hectares in area, and about 6800 smaller lakes (no further size classes were given). The numbers for the 5 smallest categories on Table I are similar, averaging 1254±140 per group, although the combined surface areas of the 5 groups account for only 40% of the total surface area. Thus while the two largest categories make up only 6% of the total number of lakes, they account for 60% of the total surface area.

Table I Numbers and areas of size classes of lakes in Nova Scotia.

Size class (ha)	Number	%	Total area (ha)	%
1-2	1339	20.1	2159	1.0
2-5	1436	21.5	5375	2.4
5-10	1163	17.4	8912	4.0
10-25	1249	18.7	20593	9.1
25-100	1081	16.2	53084	23.5
100-500	367	5.5	71781	31.8
over 500*	39	0.6	63591	28.2
Total	6674	100	225495	100

*The largest is Lake Rossignol (15470 ha).

Table II lists the number of lakes in the 44 drainage basins that are greater than 1 hectare in area. The total area of these lakes and the proportion underlain by granites or metamorphics is also given in Table II. This Table indicates that of the 6674 lakes in the province (2255 km^2), 5213 (1915 km^2) are likely to be found on granite or metamorphic rocks. Thus 78% of the total number and 85% of the total area of Nova Scotian lakes would, in absence of moderating influences of surficial geology, be susceptible to acidification. Granitic and metamorphic influences are extensive in the province, and these resistant rocks form the bedrock geology of 65% of the province.

Data for the lake pH classes (Kelso *et al.* (1986), are shown in Table III with an additional class (pH < 5) as a further indicator of acidified lakes (cf. Watt 1981). Our estimates for both total number and area of lakes in various pH classes are provided in the upper rows. Estimates of numbers only were all that were available in sub-classes of the 3 other references. Kelso *et al.* (1986) estimated that 47.3% of the lakes in this

Table II Number and area of lakes in each of 44 drainage basins, and the percentage of land area on granite or metamorphic bedrock.

Basin (Fig 1)	# Lakes	Area Lakes ha	% Granite Metamorph.	Basin	# Lakes	Area Lakes ha	% Granite Metamorph.
DMnw	17	144	0	EL	452	9923	100
DM	20	253	0	EK	390	9227	83
DN	41	555	18	EJ	372	7073	100
DO	28	415	11	EH	201	6849	100
DP	48	584	17	EG	137	5738	100
DQ	20	147	62	EF	193	8785	100
DR	45	681	19	EE	273	16155	100
DS	37	637	0	ED	307	32989	100
FA	45	816	11	EC	133	8404	100
FB	27	5873	20	EB	112	8117	100
FC	45	295	83	EA	287	17563	100
FD	197	1539	88	DA	100	4106	100
FE	105	789	81	DAli	2	6	100
FF	33	271	50	DB	118	7891	100
FG	28	117	24	DC	180	5751	75
FJ	467	12655	54	DD	41	5311	66
FH	125	3281	45	DE	174	5849	88
FHim	26	650	0	DF	33	404	12
ER	82	1507	0	DG	203	5792	35
EQ	441	6482	74	DH	78	404	15
EP	124	1855	80	DJ	28	397	45
EO	234	4313	41	DK	19	113	4
EN	283	6409	100	DL	22	457	18
EM	301	7922	100	TOTAL	6674	225494	

DMnw: A small area northwest of DM, which flows into New Brunswick.
FHim: Isle Madame.
DAli: Long Island.
There are 5213 lakes on granite or metamorphic bedrock.

Table III Estimates of acidity in 6674 lakes in Nova Scotia.*

Number of Lakes measured	Number of Lakes with Acidities in the pH ranges > 6.6	6.6-6	6-5	5-4.7	< 4.7
6674 (2255)	2065 (450)	1947 (512)	2103 (970)	364 (223)	195 (100)
6585 (2650)	441	1291	---	---	3115**
	474	727	2870	1135	1468 #
	1768	571	3565	570	200 [$]

*The sum of the areas (in km^2) of the lakes in each pH range are given in parentheses; ** Kelso et al. (1986); # estimated from Jeffries et *al.* (1986); [$] estimated from Underwood et *al.* (1986).

province have pH < 4.7 but the isopleth maps have led us to conclude that only 3% of lakes may exist in that state. Similar extrapolation of data from the 234 lakes examined by Underwood et *al.* (1986) to the 6674 lakes, gave a value of 3% (Table III). Jeffries et *al.* (1986) had no pH class < 4.7 and the number in Table III was extrapolated from their estimates for other pH classes giving a value of 22%. We believe that differences in the estimates result from inadvertant selection of varying proportions of lakes on different geological substrata. None of the studies examined included more than 3.5% of the lakes, and Kelso *et al.* (1986) only had at their disposal a data base of 1.1% of the freshwater resources of the province.

Table III shows that 80% of the surface area of lakes in the province has less than 50 μeq L^{-1} of alkalinity, and that there are somewhere between 14% and 57% of total lake area with zero alkalinity. We employ a simplified definition of alkalinity where Alk = $HCO_3^- - H^+$. When Alk is set to 0, then $H^+ = HCO_3^-$, and based on equation 1, this would occur at pH 5.5. This yielded an estimate that 26% of lake area in the province had no alkalinity (16% of the number of lakes), a condition rendered doubly critical by rapid flushing rates in many lakes, and a resultant tendency for water quality in lakes to resemble precipitation (Underwood *et al.* 1987). If only lakes on granite or metamorphic bedrock are considered, the proportion of zero-alkalinity lakes rises slightly to 21% (based on numbers) and 31% (based on areas). We estimate similarly that 88% of granitic/metamorphic lakes (95% based on areas) have less than 50 μeq L^{-1} alkalinity.

The acid neutralizing capacity in a lake is governed by a variety of internal and external processes (Schindler *et al.* 1986). It is therefore possible to find two lakes very close together which have different levels of acidity (Kerekes *et al.* 1982). Although Nova Scotia is very sensitive to acidic precipitation (Underwood *et al.* 1987), our data imply that there are fewer acidic lakes in the province than estimated by Kelso *et al.* (1986) or Jeffries *et al.* (1986). We encourage more research on watershed processes for there is still uncertainty about relative sources and sinks for acidity in Nova Scotian lakes (Gorham *et al.* 1986).

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TAXONOMIC CONSIDERATIONS OF THE GENUS *RHODOPHYSEMA* AND THE RHODOPHYSEMATACEAE FAM. NOV. (RHODOPHYTA, FLORIDEOPHYCIDAE)[1]

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Recent investigations of *Rhodophysema*, together with a literature review of the Acrochaetiales-Palmariales complex, indicate that the genus *Rhodophysema* is not closely allied either with the Acrochaetiaceae or the Palmariaceae. Accordingly, a new family, Rhodophysemataceae, is proposed. In this treatment Rhodophysemataceae is differentiated from Acrochaetiaceae by the absence of a carposporophyte and monosporangia, presence of a unique sporangial stalk cell integral to the sexual cycle and an abundance of cellular fusions. Rhodophysemataceae is separated from Palmariaceae by the occurrence of B-phycoerythrin I, presence of *Rhodophysema*-like tetrasporangia and heteromorphic sexual life histories. To date the placement of *Rhodophysema* in the Acrochaetiales or Palmariales has been based either on vegetative morphology and phycoerythrin type or on interpretations of the evolution of the tetrasporangium and life-history type. Further evidence will be required to resolve the ordinal position of this new family. We, therefore, leave Rhodophysemataceae provisionally in the Palmariales while recognizing its uncertain taxonomic position within the Acrochaetiales-Palmariales complex. The Rhodophysemataceae may indeed warrant ordinal status as new taxonomic criteria become established. The genera *Coriophyllum, Halosacciocolax, Pseudorhododiscus* and *Rhodophysemopsis* are also discussed with respect to their taxonomic placement in the Acrochaetiales-Palmariales complex.

Des études récentes de *Rhodophysema* ainsi qu'une revue des oeuvres publiées du complexe Acrochaetiales-Palmariales démontrent que le genre *Rhodophysema* n'est pas apparenté de près à aucune ordre mentionée ci-dessus. En conséquence on propose une nouvelle famille, Rhodophysemataceae. De cette façon les Rhodophysemataceae se différencient des Acrochaetiales par l'absence d'un carposporophyte et de monosporanges, ainsi que par la présence d'une seule cellule constituant la tige sporangique et faisant partie intégrante du cycle sexuel, et par une abondance de fusions cellulaires. Les Rhodophysomataceae se séparent des Palmariaceae par la présence de B-phycoérythrine I, de tetrasporanges ressemblant ceux de *Rhodophysema*, et par les développements hétéromorphiques. Jusqu'à présent l'incorporation de *Rhodophysema* dans les Acrochaetiales ou Palmariales a été fondée soit sur la morphologie végétative et le type de phycoérythrine soit sur les interprétations de l'évolution du tetrasporange et du type de cycle biologique. On a besoin de faits nouveaux pour résoudre le placement de cette nouvelle famille dans une ordre quelconque. Nous laissons alors les Rhodophysemataceae provisoirement avec les Palmariales tout en prenant conscience de leur position taxonomique incertaine dans le complexe Acrochaetiales-Palmariales. Il se peut bien que les Rhodophysemataceae méritent un rang ordinal quand de nouveaux critères taxonomiques deviendront établis. On discute aussi des genres *Coriophyllum, Halosacciocolax, Pseudorhododiscus,* et *Rhodophysemopsis* quant à leur placement taxonomique dans le complexe Acrochaetiales-Palmariales.

Introduction

Kylin (1956) placed *Rhodophysema* Batters (1900) [as *Rhododermis* Crouan frat. ex J. Agardh (1851)] in the family Squamariaceae of the Cryptonemiales. This illegitimate family name was subsequently replaced by Peyssonneliaceae Denizot (1968). Denizot did not include *Rhodophysema* in his new family and left it *incertae sedis* in the

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Florideophycidae. Until recently, most authors either have followed Denizot or have placed *Rhodophysema* in the Peyssonneliaceae.

Guiry (1974) initially proposed the family Palmariaceae to include algae having generative sporangial stalk cells, in which female reproductive structures and the carposporophyte generation were unknown. *Palmaria* Stackhouse, *Halosaccion* Kützing and *Leptosarca* Gepp & Gepp were transferred to Palmariaceae which was retained in the Rhodymeniales. Ganesan and West (1975) suggested that *Rhodophysema elegans* (Crouan frat. ex J. Agardh) P. Dixon be included in the Palmariaceae, as only male and tetrasporangial plants had been reported. In contrast, South and Whittick (1976) suggested that *Rhodophysema* was atypical of Palmariaceae owing to the lack of morphologically distinct stalk cells. Guiry and Irvine (Guiry, 1978) later raised the Palmariaceae to ordinal status. van der Meer and Todd (1980) observed microscopic crusts with sessile carpogonia in cultures of *Palmaria palmata* (Linnaeus) Stackhouse and demonstrated the presence of a type of sexual cycle which lent support to the ordinal status for Palmariales. Subsequently, *Rhodophysema odonthaliae* Masuda & Ohta (as *R. elegans*) was noted as possessing generative stalk cells and sessile carpogonia and was placed in the Palmariaceae, Palmariales by DeCew and West (1982). The type of sexual life history which they reported has been confirmed for Atlantic isolates of *R. elegans* (Saunders *et al.*, 1989), but the proposed homology of the diploid *Rhodophysema*-stalk cell with the morphologically distinct stalk cell of *Palmaria* remains speculative. Silva (1982) disagreed with the placement of *Palmaria* and *Rhodophysema* in the same family or even in the same order. Rather he regarded the putative loss of both tetrasporophyte and carposporophyte in *Rhodophysema* as being a major phylogenetic shift compared to the loss of only the carposporophyte in the Palmariales. Hawkes and Scagel (1986) placed *Rhodophysema* provisionally in the Palmariales and suggested that the genus warranted establishment of a separate family regardless of ordinal position.

Magne (1982) suggested that new taxonomic criteria were required to clarify the affinities of the *Rhodophysema* life history and the taxonomic placement of *Rhodophysema*. Glazer *et al.* (1982) placed *Rhodophysema* in Acrochaetiales Feldmann (1953, see Garbary and Gabrielson, 1987) based on the presence of B-phycoerythrin. Guiry (1987) strongly opposed placement of *Rhodophysema* in the Acrochaetiales, arguing that the structure and reproduction of the type species, *R. georgii*, is similar to those of the Palmariales. Saunders and Bird (1989), however, did not find a sexual cycle for *R. georgii*, consistent with that of the Palmariales. We have found B-phycoerythrin I to be present in *R. georgii* (unpubl. observ.).

Similarities between Acrochaetiales and Palmariales have often been noted (e.g. Irvine and Guiry, 1983, DeCew and West, 1982, Guiry, 1987). DeCew and West (1982) proposed separating the two orders on the basis of uniseriate vegetative filaments and the absence of secondary pit-connections in Acrochaetiales. Guiry (1987) likewise reported the absence of secondary pit-connections and further noted an absence of cell fusions in the Acrochaetiales. Two exceptions, *Rhodochorton concrescens* Drew and *Rhodochorton spetsbergensis* (Kjellman) Kjellman, to these criteria will be considered in a future publication.

The Rhodophysemataceae fam. nov. is proposed to contain the genera *Rhodophysema, Halosacciocolax* Lund and *Rhodophysemopsis* Masuda. Additional genera of uncertain affinity within this complex are discussed along with a consideration of the taxonomic affinities of Rhodophysemataceae within the Acrochaetiales-Palmariales complex.

Family Rhodophysemataceae fam. nov.

Thallus e filis uniseriatis compositus, aliis erectis aliis prostratis. Fila prostrata fusione cellularum coalescentia, plerumque discum basalem monostromaticum continuum formantia. Fila erecta singulatim vel fasciculatim surgentia, saepe mucilagine lutinata vel fusione cellularum coalita, pseudoparenchyma formantia. Chloroplasti numerosi, parietales, taeniati vel disciformes, pyrenoidibus carentes, pigmento B-phycoerythrino I praediti. Gametophytum monoecum, carpogonia sessilia et spermatangia geminata praebens. Tetrasporophytum continuo e carpogino fecundato ortum, maturum e tetrasporangio cruciatim diviso et cellula suffultoris constitutum. Propagatio nonsexualis bisporis vel tetrasporis mitotice formatis effecta. Galerum obturamenti intercellularis distromaticum.

Uniseriate filaments in erect and prostrate axes with prostrate axes adhering laterally by means of cell fusions usually forming a compact monostromatic base. Erect filaments arising individually or in clusters, sometimes becoming adherent and pseudoparenchymatous owing to cell fusions or mucilaginous envelopes or both. Chloroplasts parietal and ribbon-shaped or numerous and discoid; pyrenoids absent; B-phycoerythrin I present. Pit-plug with two cap layers. Gametophyte monoecious with sessile carpogonia and paired spermatangia. Fertilized carpogonium giving rise directly to the tetrasporophytic generation, *in situ* on the gametophytic generation, and consisting of a tetrasporangium subtended by a stalk cell exclusive of the sporangial wall. Asexual life history by formation of mitotic bisporangia or tetrasporangia.

Type genus: **Rhodophysema** Batters (1900, p. 377).

The description for this genus is given by Batters (1900), Irvine and Guiry (1983) and Hawkes and Scagel (1986). Saunders et *al.* (1989) have demonstrated that both sexual and asexual populations occur.

Type species: **Rhodophysema georgii** Batters (1900, p. 377).

This alga has B-phycoerythrin I (Saunders and McLachlan, unpubl.) and a direct life history by mitotic tetrasporangia (Saunders and Bird, 1989). Other species of *Rhodophysema* are discussed elsewhere (Saunders et *al.* 1989).

Other genera: Rhodophysemopsis laminariae Masuda (1976a) is composed of loosely adherent erect filaments arising from a compact basal disc. It is very similar to *Rhodophysema* and spermatangia are reported on plants containing *Rhodophysema*-like tetrasporangia. We believe that this species belongs in the Rhodophysemataceae.

Halosacciocolax kjellmanii Lund (1959) which has been variously placed in the Acrochaetiales (Cabioch and Guiry, 1976) or Palmariales (Pueschel and Cole, 1982) appears to belong in the Rhodophysemataceae. Tetrasporangia seem to be *Rhodophysema*-like (Pueschel and Cole, 1982) and spermatangia are reported to occur on the same crusts as the tetrasporangia (Lund, 1959). Hawkes and Scagel (1986) suggested that this alga may have a *Rhodophysema*-type life history. This plant may (Hawkes and Scagel, 1986) or may not (DeCew, 1983) be the only genus in the family having secondary pit-connections. The ontogeny of connections with host cells requires reinvestigation before this point can be clarified.

Uncertain genera: Pseudorhododiscus Masuda (1976b) could be placed in the Rhodophysemataceae based on its original description. Masuda reported an absence of sterile filaments around tetrasporangia, but his figures show the presence of sterile filament-like structures (Masuda, 1976b: Figs. 2 & 3). These filaments may prove to be homologous to the sterile filaments commonly reported in Rhodophysemataceae and Palmariaceae. However, Masuda reported irregular or tetrahedrally divided tetrasporangia and separate male and tetrasporangial plants. This plant needs reinvestigating and may be related to the genus *Coriophyllum* Setchell & Gardner.

Coriophyllum Setchell & Gardner (1903) possesses two-layered pit-plug caps (Pueschel and Cole, 1982) and appears to be allied with the Palmariales. DeCew (1983) suggested that the life history of *C. expansum* is similar to that of *R. elegans*. Guiry (1987), however, described the life history as being intermediate between the *Rhodophysema*-type and the *Palmaria*-type. *C. expansum* has R-phycoerythrin

(Glazer et al. 1982), is vegetatively quite different from *R. elegans,* has a dioecious gametophytic generation and extensive tetrasporophyte development on the female gametophyte (DeCew, 1983). We, conclude that *C. expansum* has its closest affinities with the Palmariaceae but requires reinvestigation and may, along with *Pseudorhododiscus,* belong in a new family. For now, *C. expansum* remains *incertae sedis* in the Palmariales as suggested by DeCew (1983).

Taxonomic Placement of the Rhodophysemataceae

We now consider the taxonomic placement of Rhodophysemataceae in relation to the families Acrochaetiaceae and Palmariaceae of the Acrochaetiales-Palmariales complex. The following characters, applicable to diagnoses of Acrochaetiales and Palmariales and Rhodophysemataceae, are discussed below: 1) vegetative morphology; 2) cell fusions and secondary pit-connections; 3) pit-plug ultrastructure; 4) phycoerythrin type; 5) tetrasporangial-type and presence of a generative stalk cell; 6) sessile carpogonia; 7) life-history type and apparent loss of tetrasporophytic and carposporophytic generations.

Vegetative morphology Filamentous Acrochaetiales have been traditionally separated from pseudoparenchymatous Palmariales. Rhodophysemataceae are pseudoparenchymatous, suggesting affinities with Palmariales. However, it is difficult to determine whether the pseudoparenchymatous condition in Rhodophysemataceae is homologous or analogous to that in Palmariales. Reports of intercalary divisions in *Coriophyllum* (DeCew, 1983) and *Rhodophysema* (Saunders et *al.*, 1989) require further investigation.

Cell fusions and secondary pit-connections Occurrence of cell fusions and secondary pit-connections has not previously provided a satisfactory basis for separating Acrochaetiales from Palmariales despite claims to the contrary (e.g. DeCew and West, 1982). Cell fusions have been reported in *Rhodochorton spetsbergensis* and *R. concrescens* (Acrochaetiaceae) and in the Palmariaceae and Rhodophysemataceae. Acrochaetioid algae that exhibit cell fusions are probably related to the Rhodophysemataceae. With the removal of the anomalous species (to be proposed in a future publication), cell fusions become a useful character for distinguishing Acrochaetiaceae from Rhodophysemataceae and Palmariaceae. Cell fusions have probably evolved numerous times in the red algae and this feature does not, in itself, imply close phylogenetic relationships between Rhodophysemataceae and Palmariaceae.

Algae placed in the Acrochaetiales are reported to lack secondary pit-connections (Guiry, 1987), although reported for one species of Palmariales, *Halosaccion americanum* I.K. Lee (Mitman and Phinney, 1985). One of us (GWS) has reinvestigated the *H. americanum* preparations of Mitman and Phinney and observed no evidence of secondary pit-connections, although an abundance of cell fusions was noted. Indeed, all reports of secondary pit-connections may be erroneous for non-parasitic Palmariales. Pueschel (1988) has suggested that algae having two-layered pit-plug caps lack secondary pit-connections and can be separated from a larger group of florideophycean algae based on this combination of characters. Garbary et *al.* (1983) noted an exceptional case of a secondary pit-connection in the Acrochaetiales, but did not elucidate the ontogeny of the structure and this too may represent a cell fusion. *Halosacciocolax* Lund and *Neohalosacciocolax* Lee & Kurogi were placed in Acrochaetiales and Palmariales respectively owing to the presence of secondary pit-connections in *Neohalosacciocolax* (DeCew, 1983) and their absence in *Halosacciocolax*. This requires clarification as pit-connections between parasite and host cells are included in the description of *Halosacciocolax* (see Hawkes and Scagel, 1986). The ontogeny of connections between *Halosacciocolax* and host cells must be elucidated to determine if these are indeed homologous to those reported for *Neohalosacciocolax* and its host, and whether connections observed in these algae are homologous to secondary pit-connections observed in the remainder of the Florideophycidae.

We consider that the Acrochaetiales and Palmariales are separable, based on the occurrence of cell fusions in only the Palmariales, pending taxonomic placement of the Rhodophysemataceae. Further, we believe that the Acrochaetiales lack, and the Palmariales may lack secondary pit-connections except for analogous structures that link parasite to host tissue. This problem has been discussed recently by Pueschel (1988).

Pit-plug ultrastructure Pueschel and Cole (1982) observed two-layered pit-plug caps in *Palmaria,* which they interpreted as being characteristic of the Palmariales. *Rhodophysema* and *Halosacciocolax* were placed in the Palmariales owing to the presence of pit-plug cap layers similar to those in *Palmaria* (Pueschel and Cole, 1982). However, they also reported two-layered pit-plug caps in Acrochaetiales which they described as being similar in both morphology and development to those in Palmariales. Therefore, this character cannot currently be used for separating Acrochaetiales from Palmariales and does not affect placement of the Rhodophysemataceae.

Phycoerythrin type *Rhodophysema* was placed in Acrochaetiales owing to the presence of B-phycoerythrin observed in *R. elegans,* compared to the Palmariales which possess R-phycoerythrin (Glazer et *al.*, 1982). Guiry (1987) correctly noted that *Rhodophysema* can be moved to the Acrochaetiales only on the basis of features observed in the type species. We investigated *R. georgii,* the type species of *Rhodophysema,* and confirmed the presence of B-phycoerythrin I (unpubl. observ.). Rhodophysemataceae is characterized solely by B-phycoerythrin, as reported for two species of *Rhodophysema,* while Palmariaceae exclusively exhibits R-phycoerythrin (Glazer et *al.*, 1982). The Acrochaetiaceae includes species with either B- or R-phycoerythrin. It is our view that phycoerythrin will eventually prove invaluable for infraordinal classification within Acrochaetiales, probably leading to the establishment of additional families.

Tetrasporangial type Two distinct tetrasporangial types, traditionally recognized in the Acrochaetiales-Palmariales complex, are referred to as the Acrochaetiales-type and the Palmariales-type, the latter probably having evolved from the former (Guiry, 1978). In the Acrochaetiales-type a stalk cell external to the sporangial wall divides, with the terminal daughter cell developing into a primary tetrasporocyte or, if secondary, enlarging within the sporangial wall to form a secondary tetrasporocyte. Repeated tetrasporocyte formation results in continued accumulation of successive wall layers (Hawkes and Scagel, 1986). In the Palmariales-type, the initial divides unequally, yielding a smaller generative stalk cell and a larger terminal tetrasporocyte (Guiry, 1978). Regeneration occurs by growth of the stalk cell into the space occupied by the original sporangial wall, followed by an unequal division. As a result, wall accumulation does not occur and the sporangial wall encloses the stalk cell and tetrasporocyte (Pueschel, 1979). This latter type occurs exclusively in the Palmariaceae. Guiry (1987) suggested that the Acrochaetiales-type stalk cell is the least specialized and is morphologically similar to a vegetative cell.

Based on our recent observations in *Rhodophysema* (Saunders and Bird, 1989: Saunders et *al.*, 1989) we consider tetrasporangia in the Rhodophysemataceae to be different from either of the two previous types as the stalk cell is diploid in sexual populations with a homologous structure present in asexual populations. The presence of intercalary stalk cells and the similarity of stalk cells to vegetative cells in the asexual populations may explain the reported absence (Hawkes and Scagel, 1986) of this structure in some species of *Rhodophysema* (see Saunders and Bird, 1989).

Tetrasporangia of *Rhodophysema*, like those of the Acrochaetiales, are distinct from the Palmariales-type in that the generative stalk cell is external to the sporangial wall and sporangial wall accumulation occurs only in the former (Guiry, 1978; 1987: Hawkes and Scagel, 1986; Pueschel, 1979). G. Mitman (*in verb.*), however, noted an accumulation of sporangial walls in *Halosaccion americanum,* which was confirmed (GWS) by a re-examination of his slides. Nevertheless, both the generative stalk cell and the tetrasporocyte were enclosed within the sporangial wall. In view of Mitman's observations, the only distinguishing character separating tetrasporangia of Acrochaetiales and *Rhodophysema* from those of Palmariales is the inclusion of the generative stalk cell within the tetrasporangial walls in the Palmariales. We consider these three tetrasporangial types as being distinct and useful characters for separating the three families.

Sessile carpogonia Sessile carpogonia are considered to have limited taxonomic value. They are present in Acrochaetiales, Palmariales and Rhodophysemataceae, and as they do not delineate the two orders they cannot be used to determine ordinal placement of Rhodophysemataceae. Although not restricted to these taxa, sessile carpogonia appear to represent an ancestral character and may be useful for recognizing taxa little evolved from the ancestral line of the Florideophycidae.

Life history type and putative loss of carposporophytic or tetrasporophytic generation Life histories are basically isomorphic in Palmariales and both heteromorphic and isomorphic in the Acrochaetiales (Gabrielson and Garbary, 1987). In reality the distinction is less pronounced as a variety of life history patterns occur in the Acrochaetiales: 1) triphasic *Polysiphonia*-type as exemplified by *Colaconema botryocarpa* (see Woelkerling, 1970); 2) an analogue of the *Rhodophysema*-type (see diagnosis) in *Rhodochorton subimmersum* Setchell & Gardner (Lee and Kurogi, 1978); 3) an analogue of the *Palmaria*-type (van der Meer and Todd, 1980) in *Rhodochorton purpureum* (Lightfoot) Rosenvinge and *Rhodothamniella floridula* (Dillwyn) J. Feldmann (Stengenga, 1978). The *Polysiphonia*-type life history in the Acrochaetiales is distinct from the *Rhodophysema* and *Palmaria* analogues. Magne (1982) suggested that the *Rhodophysema* and *Palmaria* patterns represented either phylogenetic relatedness or evolutionary convergencies. Whether the *Rhodochorton* analogues are in fact homologous to the *Palmaria*- and *Rhodophysema*-types remains to be resolved. In any event, Magne was certain that both life histories were derived from the ancestral *Polysiphonia*-type. Magne's view reflects Feldmann's opinion (1952) that a triphasic life history with free-living isomorphic gametophytic, carposporophytic and tetrasporophytic generations was primitive.

West and Hommersand (1981) were uncertain whether biphasic or triphasic life histories were primitive in the Florideophycidae. Guiry (1987) suggested that the biphasic life history noted in the Palmariales and the genus *Rhodophysema* evolved independently of other red algal life histories. He postulated that the ancestral red alga was biphasic and that evolution proceeded through two different modes of zygote amplification: 1) formation of a carposporophytic generation *in situ* on the female gametophyte; 2) the *Palmaria*-type life history. Hence, in Guiry's view, the carposporophyte was never a free-living generaton. Guiry, further, was of the opinion that the *Palmaria*- and *Rhodophysema*-type life histories evolved from a biphasic ancestor, not from an alga with the *Polysiphonia*-type life history. Current life-history data are of only limited value for establishing phylogenetic relationships within the Acrochaetiales-Palmariales complex. Attempts to assign Rhodophysemataceae to either order based on life history is speculative and unsupported by factual evidence. All three families are characterized asexually by the production of mitotic bisporangia and tetrasporangia; however, the production of monosporangia, although not ubiquitous, occurs solely in the Acrochaetiaceae.

Hawkes and Scagel (1986) proposed that algae having a *Rhodophysema*-type life history warrant recognition as a separate family. Silva (1982) remarked that the loss of both the tetrasporophytic and carposporophytic generations in *Rhodophysema* represented a major phylogenetic shift compared to the loss of only the carposporophyte in *Palmaria* and suggested that separate orders are justified.

Considering the evidence, we propose that Rhodophysemataceae; 1) has affinities with the Acrochaetiales-Palmariales complex; 2) is an assemblage distinct from the Acrochaetiaceae and the Palmariaceae; 3) can be placed only provisionally in the Palmariales on the basis of shared derived vegetative characters (which may or may not represent homology) and the lack of monosporangia; 4) that ordinal status may be required to represent the true phylogenetic affinities of the Rhodophysemataceae. We do not believe, that a new order is justified until additional taxonomic criteria are elucidated.

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LYTIC EFFECTS OF 3-(3′-ISOCYANOCYCLOPENT-2′-ENYLIDENE) PROPIONIC ACID ON THE CELLULOLYTIC RUMEN BACTERIA FIBROBACTER SUCCINOGENES AND RUMINOCOCCUS FLAVEFACIENS

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3-(3′-Isocyanocyclopent-2′-enylidene)propionic acid (ICEP), a metabolite of *Trichoderma hamatum*, depressed the specific growth rate of exponential phase cultures of *Fibrobacter succinogenes* S85 and *Ruminococcus flavefaciens* C94 at concentrations up to 4.5 μg mL^{-1}. At 5 to 20 μg mL^{-1} depression of growth rate was followed by cell lysis. *F. succinogenes* was more susceptible to these effects than *R. flavefaciens*. The divalent cations Ca^{2+}, Fe^{2+}, Mg^{2+} and Mn^{2+} reduced the specific rate of lysis in cultures of *F. succinogenes* treated with ICEP but only Fe^{2+} showed a similar effect in *R. flavefaciens* cultures. When cells of either species were removed from contact with ICEP their capacity to resume growth depended on the duration of prior contact with the compound. ICEP showed no mutagenic activity in the replicative killing bacterial test.

L'acide propionique 3-(3′-isocyano-cyclopent-2′-enylidene) (PICE), un metabolite de *Trichoderma hamatum*, a baissé le taux spécifique de croissance de cultures en phase exponentielle de *Fibrobacter succinogenes* S85 et de *Ruminococcus flavefaciens* C94, en concentrations jusqu'à 4.5 μg mL^{-1}. De 5 à 20 μg mL^{-1} du composé, la baisse du taux de croissance a été suivie d'une lyse de cellules. *F. succinogenes* était plus susceptible à ces effets que *R. flavefaciens*. Les cathions bivalents Ca^{+2}, Fe^{+2}, Mg^{+2} et Mn^{+2} ont réduit le taux specifique de la lyse quant aux cultures soumises à la PICE, mais le Fe^{+2} seulement a démontré un effet semblable dans les cultures de *R. flavefaciens*. Quand les cellules de chaque espèce étaient retirées de leur contact avec la PICE, leur capacité de reprendre la croissance dépendait de la durée de leur contact antérieur avec le composé. La PICE a demontré nulle activité mutagénique à la suite du test réplicatif de la mortalité des bactéries.

Introduction

Trichoderma hamatum produces several isocyanide metabolites with antimicrobial properties (Brewer and Taylor 1981) which have been implicated (Brewer et *al.* 1982) in the depression of rumen fermentation activity characteristic of ovine ill-thrift (Brewer et *al.* 1971). One of these metabolites, 3-(3′-isocyanocyclopent-2′-enylidene) propionic acid (ICEP; Fig 1), had a minimum inhibitory concentration (MIC) of < 10 μg mL^{-1} for the growth of nine strains of functionally important anaerobic rumen bacteria (Liss et *al.* 1985). The gram-negative strains tested were generally more

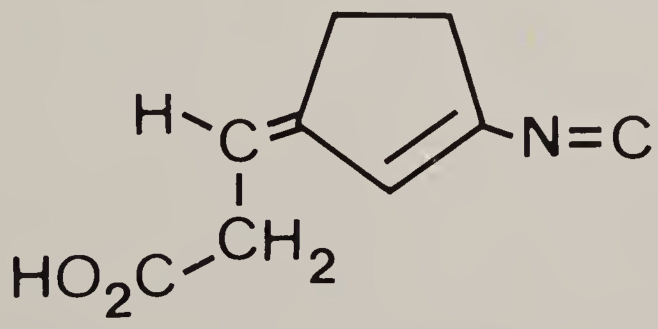

Fig 1 Structure of 3-(3′-isocyanocyclopent-2′-enylidene)propionic acid.

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sensitive than the gram-positive, and cellulolytic strains were among the most sensitive. At ICEP concentrations below the MIC the molar proportions of fermentation products produced by the strains were altered.

ICEP inhibited cellulose hydrolysis by a mixed culture of rumen microorganisms at a concentration of 2-5 μg mL^{-1} and depressed the in vitro digestion of timothy hay (Brewer *et al.* 1986). At similar concentrations it depressed the fermentation activity of strained rumen fluid, and progressively but incompletely reduced the motility of both bacteria and protozoa. The inhibition of cellulose hydrolysis, hay digestion and fermentation activity by ICEP was reversed by nickelous ion, and that of cellulose hydrolysis also by cobaltous ion. Brewer *et al.* (1986) proposed that at low concentrations ICEP inactivated nickel- and cobalt-containing cofactors, and that in the presence of supplemental Ni^{2+} or Co^{2+} compensatory cofactor synthesis supported renewed growth and metabolic activity.

When the effects of higher concentrations of ICEP on specific rumen bacteria were tested it was observed that cell lysis commonly occurred. Species representing the predominant gram-negative and gram-positive cellulolytic flora (Hungate 1966) were selected to investigate this effect and the results obtained are reported.

Materials and Methods

Isocyanide. ICEP was the gift of A. Taylor, National Research Council of Canada, Halifax, N.S. Its biological activity was standardized by means of an aerobic disc diffusion assay using *Escherichia coli* ATCC 11775 (Liss *et al.* 1985).

Bacterial Strains. Fibrobacter succinogenes Hungate (strain S85), formerly *Bacteroides succinogenes* (Montgomery *et al.* 1988), was obtained from C.W. Forsberg, University of Guelph, and *Ruminococcus flavefaciens* Sijpesteijn (strain C94) from M.P. Bryant, University of Illinois. The strains were grown anaerobically (Liss *et al.* 1985) in mSD medium (see below), and stored at -20°C after addition of 20% (vol/vol) glycerol (Teather 1982). Working stocks were maintained in the same medium and stored at 4°C.

Treatment of Rumen Bacteria with ICEP. Rumen bacteria were cultured at 39°C in the medium of Scott and Dehority (1965), modified by omission of casein hydrolysate, addition of hemin (0.0001%) and DL-2-methylbutyric acid (0.008% wt/vol), and adjustment of the cellobiose concentration to 0.5%. The modified medium had a final pH of 6.6-6.8 and is referred to as mSD medium. Cells were harvested by centrifugation under CO_2 at 10,000 x g for 10 min at 5°C and washed twice in anaerobic dilution solution (ADS; Bryant and Burkey 1953). Samples of the final suspension were used to inoculate fresh mSD medium. Cultures were incubated at 39°C to the early exponential phase of growth (0.5 - 1.0 x 10^9 cells mL^{-1}) and then distributed under CO_2 to sterile tubes (10 x 100 mm) in 3 mL volumes. When experiments involved metal ions or EDTA, the desired salts or compound were distributed to tubes as sterile, N_2-saturated solutions (0.01 mL) prior to addition of the cell suspension (2.9 mL). ICEP in N_2-saturated 1% $NaHCO_3$ (0.01 mL) was added and the cultures were incubated at 39°C. All cultures were prepared in triplicate unless otherwise indicated and appropriate controls were included in each experiment. Optical density was measured at 600 nm. To calculate rates, readings taken in the exponential phase of growth or lysis were linearized by conversion to adjusted optical density (Jones and Pickard 1980) and transformed to natural logarithms. Regression of the resulting values on time was then analyzed. The positive slope of the regression line represented specific growth rate (μ, h^{-1}); the negative slope represented the specific rate of lysis (k, min^{-1}). The kinetics of the reaction between ICEP and the bacteria were investigated by means of

Hill and Eadie-Hofstee plots; *k* was substituted for velocity and ICEP concentration for substrate concentration in each of the respective equations. To confirm cell lysis, portions of culture supernatants were washed with diethyl ether to remove ICEP and absorbance was measured at 260 nm; supernatants were also analyzed for free α-amino nitrogen (Yemm and Cocking 1955), protein (Lowry *et al.* 1951) and pentose sugars (Herbert *et al.* 1971).

Enumeration of Bacteria. Viable counts of rumen bacteria were estimated by the roll tube method (Hungate 1969) using ADS as diluent and mSD medium supplemented with 2% agar. Colonies were enumerated after incubation at 39°C for 5 days.

RK Bacterial Test. The toxicity and mutagenic potential of ICEP were determined by the RK (replicative killing) bacterial test of Hayes *et al.* (1984), using methyl-N'-nitrosoguanidine (MNG) as control mutagen. This test employs *E. coli* CHY 832, a strain in which genes carried on a small integrated genomic fragment are regulated by a temperature-sensitive protein. Mutagens increase the frequency of reversion to temperature-insensitivity; therefore the number of survivors increases when treated cells are incubated at elevated temperature.

Statistical Analysis. Analysis of variance calculations on rates of lysis of rumen bacteria derived from studies of the interaction of ICEP with metal ions and EDTA were made using Statworks software (Cricket Software Inc., Philadelphia, PA). Following analysis of variance, means were compared by Duncan's (1955) multiple range test.

Results

MICs of ICEP for Rumen Bacteria. The minimum concentrations of ICEP which inhibited the growth of *F. succinogenes* S85 and *R. flavefaciens* C94 in mSD medium for 18 h were 2 and 5 μg mL^{-1}, respectively. The biological activity of ICEP was stable for at least 7 h when the compound was incubated at 39°C in sterile mSD medium adjusted to pH values between 5.8 and 7.2.

Toxicity and Mutagenic Potential of ICEP. In the RK bacterial test ICEP was about twenty-five times less toxic to *E. coli* CHY 832 than MNG and lacked mutagenic activity.

Lytic Effects of ICEP. When ICEP at concentrations up to 4.5 μg mL^{-1} was added to cultures of *F. succinogenes* and *R. flavefaciens* in the early exponential phase of growth, μ was depressed (Table I). At 5-20 μg mL^{-1}, the growth rate was initially

Table I Effect of 3-(3'-isocyanocyclopent-2'-enylidene)propionic acid (ICEP) on specific growth rate of *Fibrobacter succinogenes* and *Ruminococcus flavefaciens*.

ICEP concentration (μg mL^{-1})	Specific growth rate (h^{-1})	
	F. succinogenes	*R. flavefaciens*
0	0.397 (0.989)[a]	0.322 (0.978)
2	0.392 (0.999)	0.093 (0.881)
4	0.298 (0.997)	0.072 (0.900)
4.5	0.198 (0.980)	0.047 (0.821)

[a] Correlation coefficient.

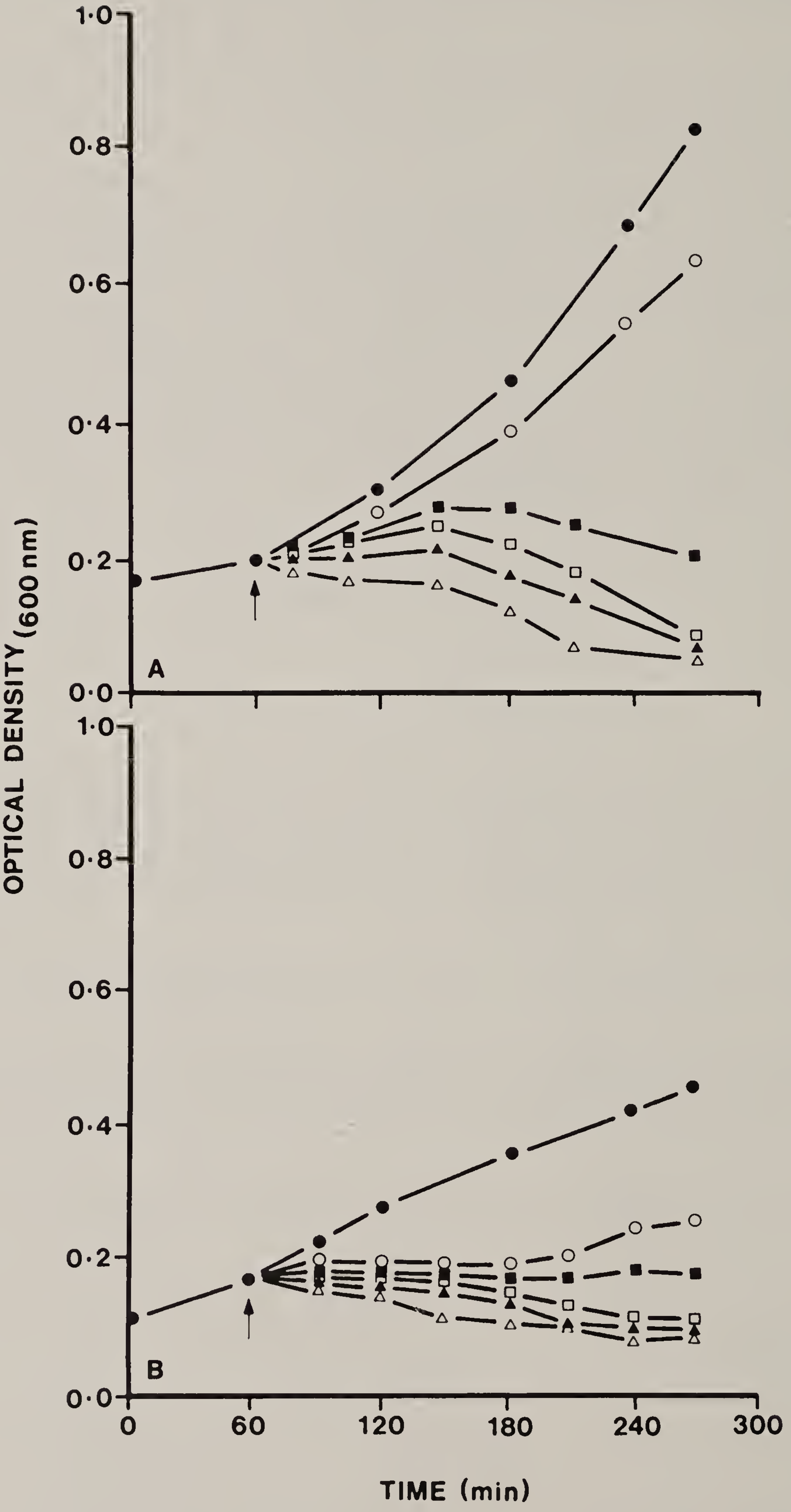

Fig 2 Effect of 3-(3′-isocyanocyclopent-2′-enylidene)propionic acid (ICEP) on growth of exponential-phase cultures of *F. succinogenes* (A) and *R. flavefaciens* (B). ICEP concentration (μg mL^{-1}): ●, 0; ○, 2; ■, 5; □, 7; ▲, 10; △, 20. Arrows indicate time of ICEP addition.

depressed or abolished and the optical density of the cultures subsequently declined (Fig 2). The higher the concentration of ICEP the more rapid the onset of these changes. These effects were generally more dramatic with *F. succinogenes* than *R. flavefaciens.* Lysis of the cultures in the presence of ICEP was confirmed by a time-dependent increase in material absorbing at 260 nm and the appearance of extracellular α-amino nitrogen, protein and pentose sugars in culture supernatants. Both species lysed, as judged by a decline in optical density, at the end of exponential growth in mSD medium lacking ICEP.

The specific rate of lysis (k) reflected the difference in susceptibility of the two species to lysis and suggested that ICEP displayed saturation kinetics; no increase in k was seen with either species at an ICEP concentration $> 20\,\mu g\ mL^{-1}$. When the data were linearized using the modified Hill equation the resulting plots (Fig 3) showed a strong positive correlation between the fraction log (k/k_{max}-k) and log [ICEP]. The positive Hill coefficients (8.18 for *F. succinogenes* and 4.69 for *R. flavefaciens*) indicate that as the ICEP concentration was increased the rate of approach of k to k_{max} was greater for the former organism than the latter by a factor of 1.7. This suggests a more extensive reaction of ICEP molecules with *F. succinogenes* than *R. flavefaciens.* The relationship k vs. k/[ICEP] indicated that saturation kinetics were displayed by ICEP activity against *F. succinogenes* but not *R. flavefaciens.* This suggests that lysis of the former organism is a primary consequence of ICEP activity and in the latter a secondary consequence.

Addition of Ca^{2+}, Fe^{2+}, Mg^{2+} and Mn^{2+}, but not Zn^{2+}, dramatically reduced k in cultures of *F. succinogenes* treated with ICEP, but only Fe^{2+} showed a similar effect in cultures of *R. flavefaciens* (Table II). Addition of EDTA to treated *F. succinogenes* cultures, however, resulted in an increase in k over the concentration range tested. These results suggest that ICEP destabilizes the outer membrane of the gram-negative species.

Recovery of Cultures Treated with ICEP. Experiments were conducted to determine whether cultures of *F. succinogenes* and *R. flavefaciens* undergoing lysis in the presence of ICEP ($10\,\mu g\ mL^{-1}$) for up to 180 min could recover if the cells were

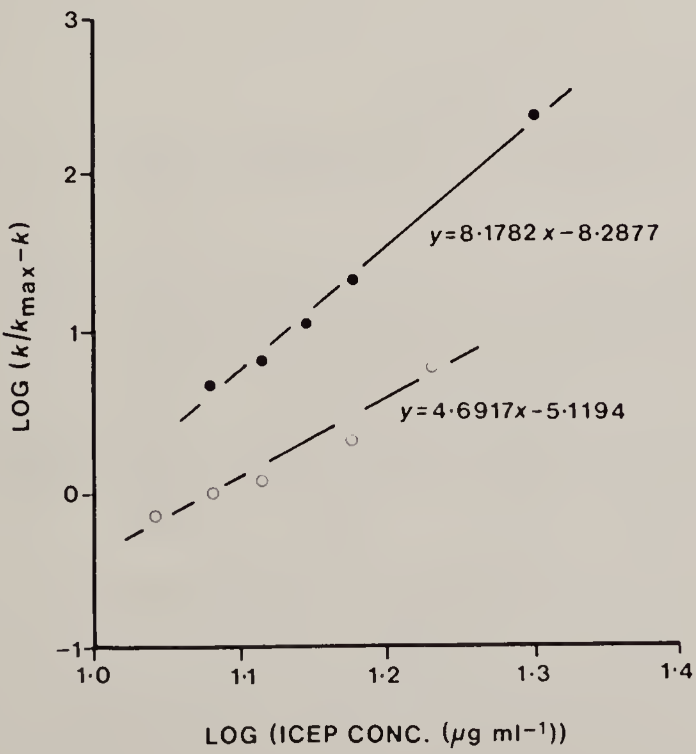

Fig 3 Effect of 3-(3′-isocyanocyclopent-2′-enylidene)propionic acid (ICEP) concentration on the specific rate of lysis (k) of *F. succinogenes* (●) *and R. flavefaciens* (○) (Hill plots).

Table II Effect of metal ions or EDTA on a specific rate of lysis (*k*) of *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* cultures treated with 3-(3′-isocyanocyclopent-2′-enylidene)propionic acid (ICEP)[a].

Treatment	Concentration in mSD medium (mM)	Supplemental concentration (mM)	*k* (min^{-1} x 10^{3})[b]	
			F. succinogenes	*R. flavefaciens*
Control (ICEP alone in mSD medium)			15.2 ± 0.3^{a}	7.4 ± 0.2^{a}
Ca^{2+}	0.45	0.06	6.8 ± 0.4^{b}	
		0.12	6.0 ± 0.4^{c}	7.2 ± 0.2^{a}
		0.24	5.6 ± 0.2^{c}	7.2 ± 0.1^{a}
Fe^{2+}	0.07	0.06	9.1 ± 0.2^{d}	
		0.12	7.4 ± 0.2^{e}	5.9 ± 0.2^{b}
		0.24	5.6 ± 0.2^{c}	4.5 ± 0.2^{c}
Mg^{2+}	0.42	0.06	10.6 ± 0.7^{f}	
		0.12	7.6 ± 0.2^{e}	7.3 ± 0.2^{a}
		0.24	2.2 ± 0.2^{g}	7.2 ± 0.2^{a}
Mn^{2+}	0.12	0.06	10.3 ± 0.9^{f}	
		0.12	8.2 ± 0.5^{e}	7.5 ± 0.2^{a}
		0.24	7.6 ± 0.2^{e}	7.2 ± 0.4^{a}
Zn^{2+}	0.07	0.06	16.4 ± 0.5^{h}	
		0.12	16.9 ± 0.3^{h}	
		0.24	16.4 ± 1.0^{h}	
EDTA		0.06	15.8 ± 0.4^{a}	
		0.12	17.1 ± 0.5^{h}	
		0.24	19.0 ± 1.2^{i}	

[a] ICEP concentration: 20 μg mL^{-1} (0.12 mM).

[b] All values are the mean of 6 replicates ± standard deviation.

[a–i] Means in a column not sharing the same letter differ significantly ($P < 0.05$).

removed to medium lacking the compound. Cultures of *F. succinogenes* resumed growth with no lag following exposure for up to 60 min but did not resume growth within the experimental period after exposure for 120 min or more (Fig 4). Cultures of *R. flavefaciens* resumed growth after all exposure times up to 180 min. In both cases the initial rate of renewed growth was inversely proportional to the duration of contact with ICEP. The capacity to recover was greater for *R. flavefaciens* than *F. succinogenes*.

Bacteriostatic and Bactericidal Effects of ICEP. To determine the bactericidal concentrations of ICEP, viable cells were enumerated in cultures exposed to increasing concentrations of the compound. Cultures initially contained 10^{8} - 10^{9} viable cells per mL. In the presence of 2 or 5 μg mL^{-1} ICEP *F. succinogenes* showed an initial lag period before the number of viable cells began to decline (Fig 5A). At 2 μg mL^{-1}, ICEP was bacteriostatic for this species but at 5 μg mL^{-1} it was bactericidal, killing 99% of the cells within 300 min. Viable cells were not recovered after exposure for 150 min and 30 min at ICEP concentrations of 20 and 30 μg mL^{-1}, respectively. At 5 μg mL^{-1}, ICEP was

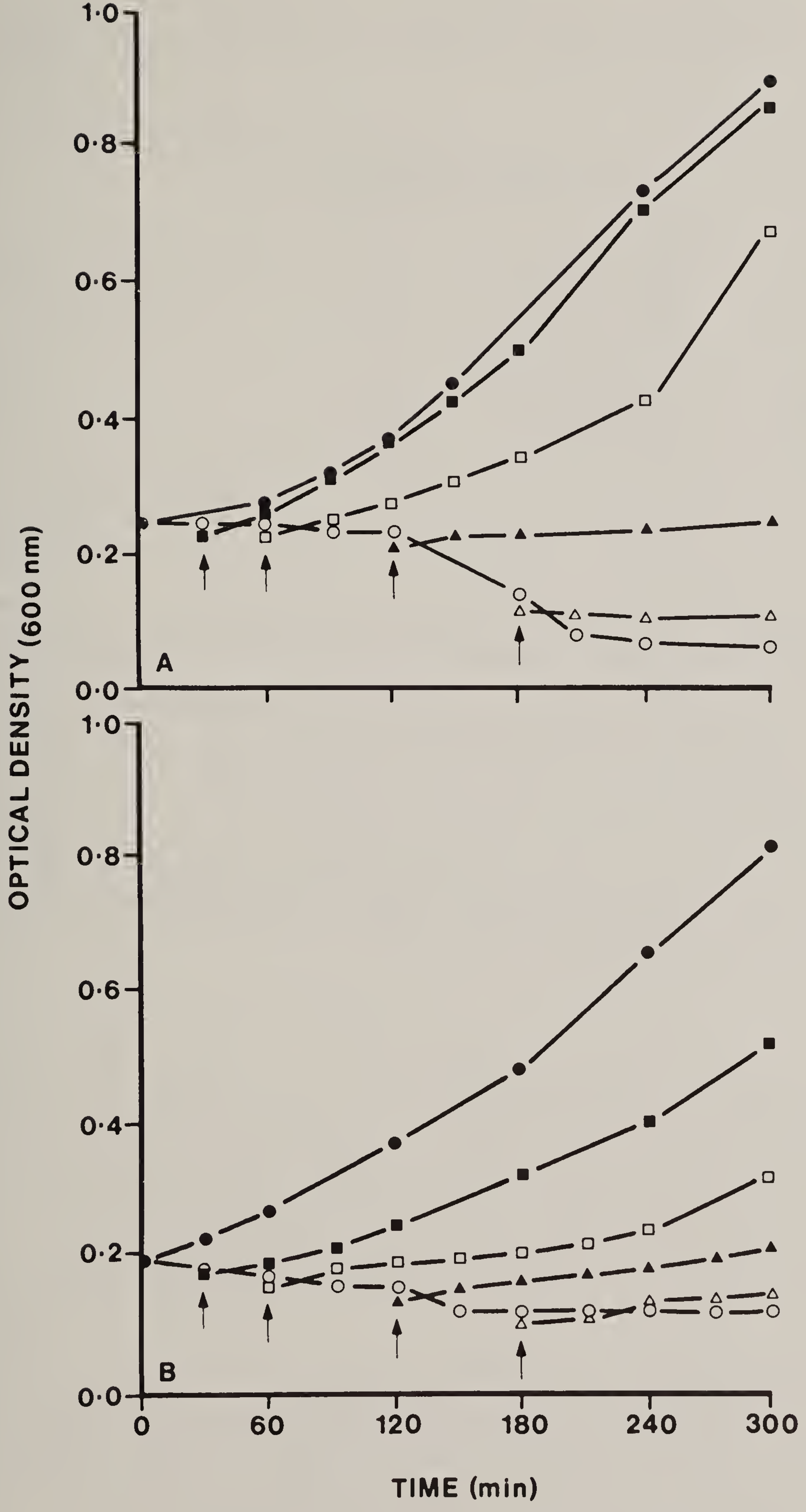

Fig 4 Effect of time of exposure to 3-(3′-isocyanocyclopent-2′-enylidene)propionic acid (ICEP) on recovery of growth of *F. succinogenes* (A) and *R. flavefaciens* (B). ICEP concentration (μg mL^{-1}): ●, 0; ○, 10. Arrows indicate times (min) at which culture aliquots were removed, washed, and resuspended in medium lacking ICEP: ■, 30; □, 60; ▲, 120; △, 180.

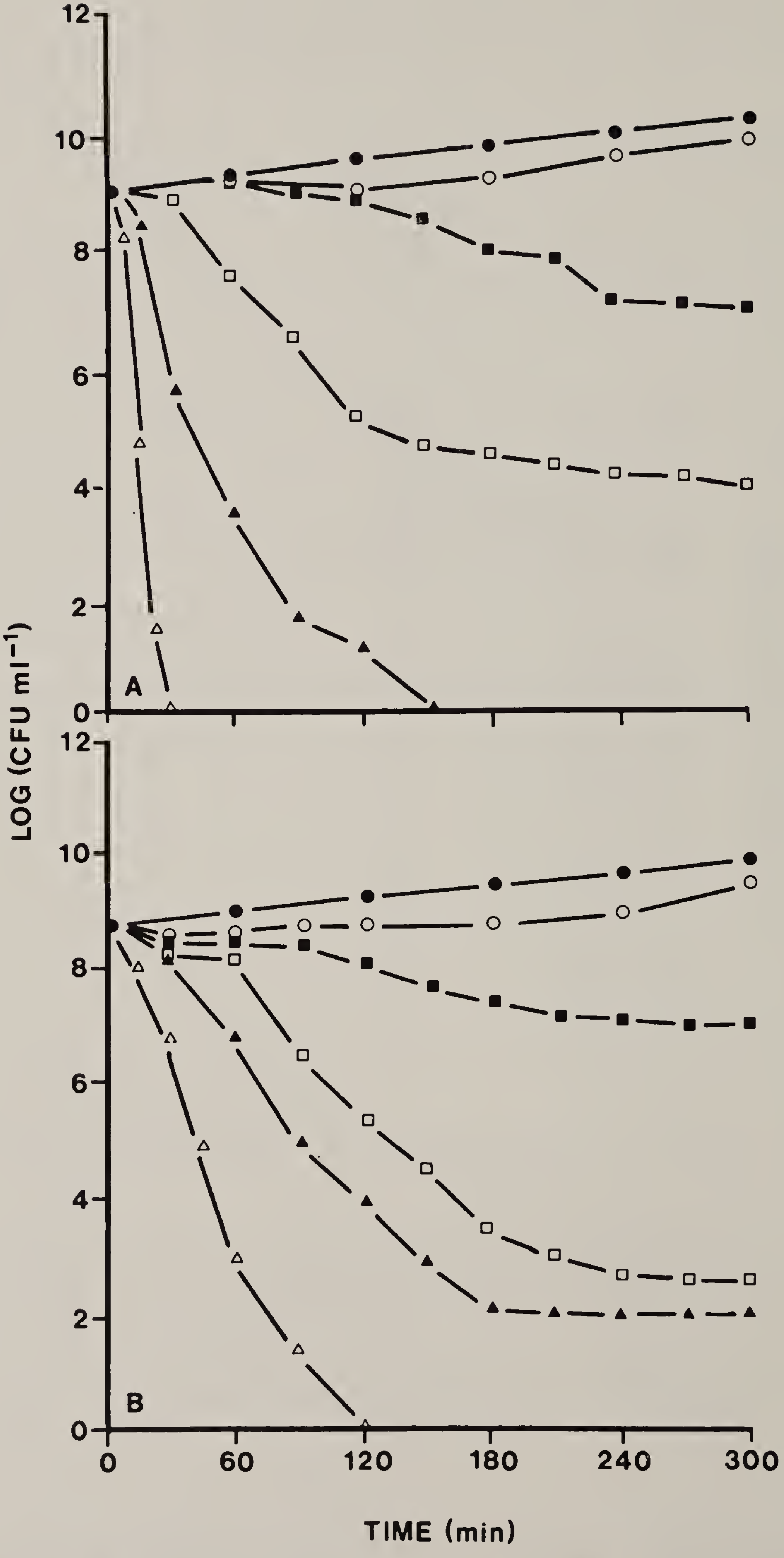

Fig 5 Effect of time of exposure to 3-(3′-isocyanocyclopent-2′-enylidene)propionic acid (ICEP) on viability of *F. succinogenes* (A) and *R. flavefaciens* (B). ICEP concentration (μg mL^{-1}): (A)●, 0; ○, 2; ■, 5; □, 10; ▲, 20; △, 30. (B)●, 0; ○, 5; ■, 10; □, 20; ▲, 30; △, 40.

Table III Regression equations relating 3-(3′-isocyanocyclopent-2′-enylidene)propionic acid (ICEP) concentration (C) and time of exposure (T) required to kill given fractions of *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* populations.

Strain	Fraction of population killed	$\log_{10}T$	Correlation coefficient
F. succinogenes	0.50	$-2.19 \log_{10} C+3.68$	-0.997[a]
	0.90	$-1.94 \log_{10} C+3.61$	-0.998[b]
	0.99	$-2.04 \log_{10} C+3.90$	-0.994[a]
R. flavefaciens	0.50	$-1.54 \log_{10} C+4.01$	-0.989[b]
	0.90	$-1.25 \log_{10} C+3.45$	-0.955[b]
	0.99	$-1.66 \log_{10} C+3.55$	-0.993[a]

[a] Significant ($P < 0.01$).
[b] Significant ($P < 0.05$).

bacteriostatic for *R. flavefaciens* but higher concentrations were bactericidal (Fig 5B). A concentration of 10 μg mL^{-1} was required to kill 99% of the cells within 300 min and one of 40 μg mL^{-1} to kill 100%.

The lag phase which occurred before viability declined in the presence of low concentrations of ICEP indicated that the kill curves were of the "multi-hit" type. These curves were used to determine [ICEP]/time relationships for 50, 90 and 99% kill (Table III). Comparison of the mean concentration coefficients (± standard error) calculated from the resulting regression equations (-2.05 ± 0.12 and -1.48 ± 0.11, respectively) indicates that ICEP was 1.3 times more effective against *F. succinogenes* than *R. flavefaciens,* a result similar to that found for the respective values of *k*.

Discussion

The results confirm previous work (Liss *et al.* 1985) showing that at low concentrations (< 5 μg mL^{-1}) ICEP inhibits the growth of cellulolytic rumen bacteria. Although at 2 μg mL^{-1} the MIC for *F. succinogenes* was 40% of that for *R. flavefaciens,* ICEP added to growing cultures had a more dramatic effect on the growth rate of the latter species than the former. Thus at 2 μg mL^{-1} the respective decreases in μ were 71% and 1%, and at 4.5 μg mL^{-1} they were 85% and 50%. However, at ICEP concentrations > 5 μg mL^{-1} lysis of the cultures was induced and *F. succinogenes* was the more susceptible organism.

Both species lysed in the stationary phase of growth in the absence of ICEP. Cultures of *F. succinogenes* grown with soluble sugars as energy source are well known to lyse following exponential growth (Gaudet 1987; Holdeman *et al.* 1984; Stewart *et al.* 1981) and other morphological changes may also occur in aging cells. For example, recent evidence (Gaudet & Gaillard 1987) suggests that the formation and release of subcellular membrane vesicles by this organism is a function of aging cells; in cellulose-grown cultures a high proportion of extracellular polysaccharidase activity may be associated with such vesicles (Forsberg *et al.* 1981). Published growth data for *R. flavefaciens* cultured in a variety of media containing cellobiose as energy source also reveal extensive lysis in the stationary phase (Pettipher & Latham 1979;

Slyter & Weaver 1977; Wachenheim & Hespell 1985). Thus both species are susceptible to lysis, probably mediated by the activity of endogenous autolysins under conditions of nutrient depletion.

At concentrations > 5 μg mL^{-1}, ICEP appeared to have a direct effect on cell envelope integrity in growing cultures of *F. succinogenes.* This organism has an envelope ultrastructure typical of gram-negative bacteria (Costerton *et al.* 1974). The interaction of ICEP with divalent cations, especially Mg^{2+}, and with EDTA suggests that it may destabilize the lipopolysaccharide component of the outer membrane. In *F. succinogenes* the outer membrane lacks strong adhesion to the underlying peptidoglycan layer (Forsberg *et al.* 1981; Gaudet & Gaillard 1987); moreover, it is a pliable structure which closely adopts the surface contours of plant tissue to which the cell adheres during digestion (Forsberg *et al.* 1981; Latham *et al.* 1978). Destabilization of the outer membrane alone, however, could not account for cell lysis because the peptidoglycan layer, although thin in this organism (Gaudet & Gaillard 1987), would be likely to maintain cell integrity (Costerton *et al.* 1974). Thus the lytic effect of ICEP on *F. succinogenes* may also require the expression of peptidoglycan hydrolase activity and perhaps that of other autolytic enzymes as well. Disruption of both the outer membrane and the peptidoglycan layer could then lead to osmotic lysis in a hypertonic medium.

Ca^{2+} and Mg^{2+} dramatically reduced k when added to *F. succinogenes* cultures at concentrations between one-half and twice that of ICEP (0.06 to 0.24 mM), even though the medium initially contained these ions at concentrations of 0.42 and 0.45 mM, respectively. This suggests that a high proportion of the ion concentrations initially present was unavailable when the bacterial suspension and ICEP were added or that extensive binding of ions to the cells occurred during growth.

Apart from Fe^{2+}, the divalent cations which modulated the lytic effect of ICEP on *F. succinogenes* did not influence its activity against *R. flavefaciens* over the concentration range tested. This reflects the gram-positive character of the ruminococcus cell wall (Pettipher and Latham 1979). Lysis in this organism also presupposes destabilization of peptidoglycan and may therefore also require the expression of endogenous peptidoglycan hydrolase activity. The availability of autolysis-defective mutants of *F. succinogenes* and *R. flavefaciens* would facilitate the testing of this hypothesis, and ICEP itself might serve as a convenient agent for selecting such mutants. Surfactant-induced lysis of *Streptococcus faecalis* has been shown to involve expression of the cellular autolytic enzyme system (Cornett and Shockman 1978).

Previous work on the inhibition by ICEP of fermentation activity in strained rumen fluid (Brewer *et al.* 1986), and results obtained in the present work, indicate that the duration of interaction between ICEP and rumen bacteria, as well as ICEP concentration, is a determining factor in the severity of bacterial response. The duration of interaction also influenced the capacity of Ni^{2+} added to strained rumen fluid after ICEP to reverse its inhibitory effect (Brewer *et al.* 1986). These results show that ICEP concentration mediates the rate at which the bacteria accumulate sufficient antibiotic to cause metabolic inhibition, death or lysis. Kinetic data satisfied the "multi-hit" hypothesis for ICEP killing, indicating that multiple target sites occur for each strain.

The effects of subinhibitory concentrations of ICEP on the production of fermentation products by pure cultures of rumen bacteria were interpreted by Liss *et al.* (1985) as showing that ICEP influenced bacterial energy metabolism. This is consistent with the conclusion of Brewer *et al.* (1986) that at low concentrations ICEP may inactivate nickel- and cobalt-containing cofactors, because such cofactors are involved in the energy metabolism of anaerobic bacteria (Gottschalk 1986). Since regulation of the onset of lysis in *Bacillus subtilis* has been linked to the energy status of the cytoplasmic membrane (Jolliffe *et al.* 1981), the lytic effect of higher concentrations of ICEP against *F. succinogenes* and *R. flavefaciens* could also be consistent with energy metabolism as the primary target of ICEP action.

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La Revue *Proceedings of the Nova Scotian Institute of Science* publie les travaux de l'Institut, des articles originaux et des notes avant trait aux sciences naturelles aux Provinces atlantiques, surtout dans les domaines de la biologie et de la géologie. Les manuscrits peuvent être en anglais ou en français, et seront soumis à l'avis d'experts avant d'être acceptés.

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STUDIES ON THE PHYTOPLANKTON AND THE WATER QUALITY OF MALPEQUE BAY, PRINCE EDWARD ISLAND, CANADA

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The phytoplankton populations of Malpeque Bay, Prince Edward Island, Canada were studied to determine the role, if any, of the water quality in realtion to the biomass, distribution and size of taxonomic groups. The phytoplankton population density was in the range 10^3 - 10^6 cells L^{-1} and 153 species were identified. Summer blooms of *Thalassiosira gravida, T. nordenskioldii, Biddulphia pulchella, B. rhombus, Chaetoceros constrictum, Dinophysis caudata* and *D. norvegica* were observed. The salinity, pH and dissolved oxygen analyses showed that the Malpeque Bay waters were of good quality. Regression analysis of the biomass data and the physico-chemical characteristics of the water indicated a positive correlation between the phytoplankton population density and the dissolved oxygen concentration, the temperature of the water, its salinity and its chlorophyll content.

Les populations phytoplanctoniques de la Baie de Malpeque, Ile-du-Prince-Edouard furent étudiées afin de déterminer le rôle, s'il y a, de la qualité de l'eau en relation avec la biomasse, la distribution et la taille des groupes taxonomiques. La densité des populations phytoplanctoniques se situait entre 10^3 - 10^6 cellules L^{-1} et 153 espèces furent identifiées. Des blooms estivales de *Thalassiosira gravida, T. nordenskioldii, Biddulphia pulchella, B. rhombus, Chaetoceros constrictum, Dinophysis caudata* et *D. norvegica* furent observées. La salinité, le pH et les analyses d'oxygène dissout démontrent que les eaux de la Baie sont de bonne qualité. Les analyses de régression des données de la biomasse et des caractéristiques physico-chimiques de l'eau indiquent qu'il y a une corrélation positive entre la densité de la population phytoplanctonique et la concentration d'oxygène dissout, la température de l'eau, la salinité ainsi que le contenu chlorophyllien.

Introduction

Malpeque Bay, Prince Edward Island is known for its shell fishery. Work on oysters (Needler, 1931, Medcof, 1961) and other invertebrates (Sullivan, 1943) has been reported from this area. The Canadian Committee of the International Biological Programme (1969-1972) conducted studies in the Gulf of St. Lawrence, and Malpeque Bay was included in this work (Steven, 1974). The annual production cycle of Malpeque Bay, and especially its primary productivity and chlorophyll variation with respect to changes in levels of nitrate and phosphate were investigated by McIver (1972). Bailey (1910, 1912, 1913a & b) reported identifications of some phytoplankton and benthic diatoms found in the coastal waters of Prince Edward Island. Benthic diatom communities colonizing plastic collectors in the Bideford River estuary of Malpeque Bay have been studied (Bacon, 1972, Bacon & Taylor, 1976). Full details of chemical analyses, taxonomy of collected organisms and seasonal changes in the main groups of phytoplankton are given in the Ph.D. thesis (Sita Devi, 1980) which has been deposited in the Library, University of Salford, Salford, Lancashire, U.K. and a copy of the same is available at the Library, Bedford Institute of Oceanography, Dartmouth, N.S., Canada.

Materials and Methods

Water samples (204, each 4 L) were collected from 22 locations (Fig 1); those near the Ellerslie Biological Station were named N1 to N6 to denote their estuarine character and to separate them from the stations in the body of Malpeque Bay which were designated M1 to M16. Samples were collected from the sites labelled N in 1973 (13) and in 1974 (N1, 10; N3, 10; and N6, 11). Samples (3) were collected in June, July and August 1973 from the M stations.

Physico-chemical analyses. Transparency was determined with a Secchi disc (20 cm diameter) and acidity with a pH meter. Salinity was measured hydrometrically and by means of a salinometer (Beckmann) against a standard sea-water sample. Analyses for dissolved oxygen, ammonia, nitrate, nitrite, Kjeldahl nitrogen, phosphate and silicate were done by standard methods. Trace metals - Cd, Hg, Cu, Fe, Mn, Zn (direct aspiration), Ni and Pb (extraction) were estimated using an atomic absorption spectrophotometer (Jarrell Ash, Model 810). Chlorophyll and phaeophytin were determined fluorimetrically (Menzel, 1963; Holm-Hansen et al., 1965) using a Turner fluorimeter.

Collection, identification and estimations of population densities of phytoplankton. Phytoplankton were collected by passing the water sample (4 L) through nets of porosity 20 μ and 40 μ. The number of phytoplankton per liter were estimated by fixing the organisms in Lugol's iodine and counting them by using an Inverted Microscope, Wild Model - 40 (Utermohl, 1958; Lund et al., 1958).

Identification of diatoms was done on permanent slides which were prepared by clearing and digesting chloroplasts using acid and hydrogen peroxide. The diatoms were mounted either in Hyrax, Pleurax or Euparol medium. The classification of Hendey (1964) for diatoms and the checklist of British Marine Algae (Parke and Dixon, 1976) for the taxonomic description of the remaining phytoplankton were used.

Species of phytoplankton were divided into three groups based on their population densities: one group, considered to be dominant consisted of species present in more than one water sample at a density greater than 10^4 cells L^{-1} and comprising more than 1% of the total population. A second group, considered to be subdominant had population densities in the range 10-5000 cells L^{-1} and comprised $<1\%$ and $>0.5\%$ of the population. The third group was the remaining species. The data were sufficiently comprehensive to allow calculations of species densities (d) and equitability (e) using the methods of Lloyd et al (1964, 1968). The effects of season and water depth on these phytoplankton groups were studied by separating the collection stations as follows: stations M1-M16 (summer, surface waters); stations N1, N3, & N6 (all seasons, surface waters); stations N2, N4, N5 (summer and autumn, surface waters); and stations N1, N3, & N6 (all seasons, bottom waters). Correlation coefficients were then calculated for comparisons of the phytoplankton populations and the physico-chemical analyses obtained on the water samples collected in these grouped locations, using the Statistical Package for the Social Sciences (SPSS) on a UNIVAC - 1100 computer - Model-60, at the Marine Environment Data Service, Department of Fisheries & Oceans, Ottawa.

Results

The physico-chemical characteristics of the water at the sampling stations are briefly summarized in Table I. The values given in Table I are the mean values obtained, with an indication of the range observation in each case. The summer surface water temperatures of the M stations (Fig 1) lay in the range 19°C to 25°C and the highest temperatures were recorded in June. The bottom water temperatures were usually ±1° of the surface temperature. Although the range of dissolved oxygen

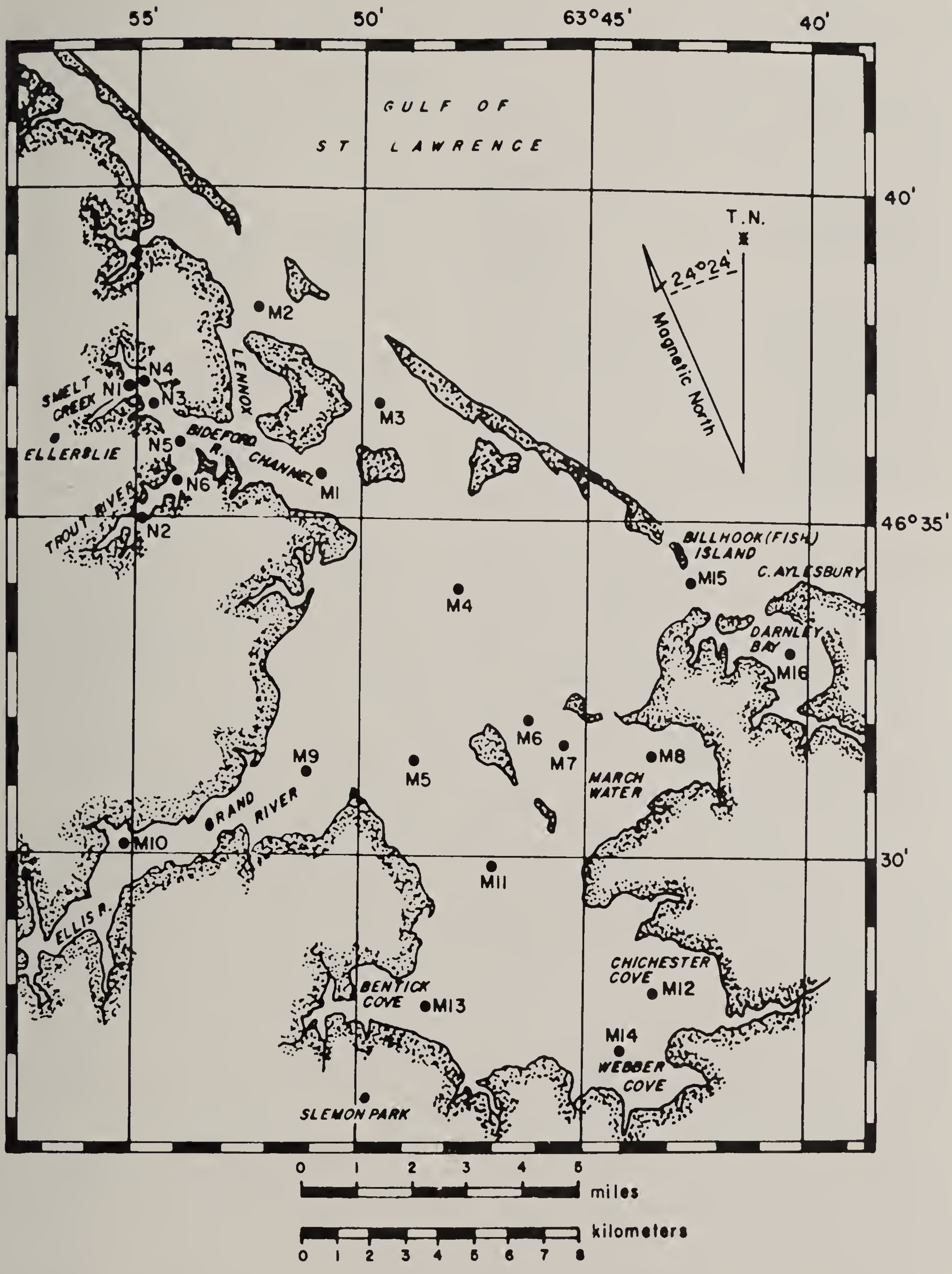

Fig 1 Map of Malpeque Bay, Prince Edward Island, showing the location of the collecting sites.

Table I Water quality characteristics of Malpeque Bay, Prince Edward Island

Characteristic	Units	Mean Value (S.D.)	Range	No. of Analysis
Stations N1-N6				
temperature	deg. C	13.3	-1.4-26	176
pH	-	8.1	7-8.9	176
salinity	0/00	24.6	0.1-27.9	176
dissolved oxygen	μg mL^{-1}	6	10.6-0.7	176
nitrogen (Kjeldahl)	μg mL^{-1}	.43 (.16)	0.14-0.79	176
phosphate	μg mL^{-1}	.04 (.02)	.009-.084	176
silicate	μg mL^{-1}	0.11	.01-.38	176
chlorophyll	μg mL^{-1}	2.9	0.1-10.7	176
Stations M1-M16				
temperature	deg. C	21.9	19.4-24.8	48
transparency	M	2.1	1.1-3.7	48
phosphate	μg mL^{-1}	.01 (.002)	.003-.013	48
silicate	μg mL^{-1}	0.014	.008-.034	48
chlorophyll	μg L^{-1}	2.01	.6-6.4	48

concentrations at the N stations varied, the variation was largely due to the lower values obtained in winter, presumably due to low photosynthetic activity. Apart from Kjeldahl nitrogen determinations, values for ammonia (2-90 ng mL^{-1}) nitrate (2-510 ng mL^{-1}) and nitrite (1-41 ng mL^{-1}) were obtained. In general the highest values for these entities were found at the N stations. A number of analyses for trace metals in the water at the N stations were obtained. The values (in ng mL^{-1}) obtained were: Ni & Zn, 10-60; Cu, Fe, Pb & Mn, 10-40; Cd, 1-6; and Hg, 1-3. These values were comparable to those reported by other workers. From all these analyses it may be concluded that the water in Malpeque Bay in 1973 and 1974 was of high quality. The low numbers of coliform bacteria found in the water samples lends support to this conclusion.

Composition and seasonal variation of the phytoplankton collected. The five orders of phytoplankton found are given in Table II together with the number of genera detected and the number of species identified in each order. The dominant species in each order are also given in Table II where in addition an indication of their seasonal variation may be found. Blooms of single species were observed in Malpeque Bay; some of these are indicated in Table II, but other species outside the dominant and subdominant classes e.g. *Biddulphia rhombus* and *Chaetoceros constrictum* were also observed to bloom in the summer. A full list of all species identified is given in Appendix I. Sixty species of benthic algae were identified; the dominant species are marked with an asterisk in Table II.

An attempt was made to calculate a species diversity index (Parsons & Takahashi, 1973) and values of 2.2-2.7 were obtained from the 1973 data from the N stations and 3-3.7 from the M stations. The greater values obtained from the M stations indicates greater biological stability (Odum, 1951).

Detailed statistical analyses of the chemical and biological data were undertaken. In particular, multiple regression analyses were carried out on the parameters of phytoplankton numbers (total numbers, reciprocal & logarithmic transforms of these numbers) and porphyrin analyses, air and water temperatures, salinity, acidity and concentrations of oxygen, phosphate, nitrogen, silicate and the trace metals Cu, Cd and Hg. The positive correlations are summarized in Table III and these results indicate, as expected, that the population density of the phytoplankton is correlated

Table II Dominant species of phytoplankton found in the waters of Malpeque Bay, Prince Edward Island and seasonal changes in their populations.

Order	No. of genera	No. of species	Dominant Species	Seasons
Baccillariophyceae	55	123	*Hyalodiscus scotius**	all
			Skeletonema costatum	all
			Biddulphia aurita	all
			*Bacillaria paxillifer**	not winter
			*Paralia sulcata**	not winter
			Chaetoceros concavicorne	not winter
			C. decipiens	not winter
			Thalassiothrix longissima	spr. & sum.
			Thalassiosira gravida	spr. & sum.
			T. nordenskioldii	spr. & sum.
			Biddulpia pulchella	spr. & sum.
			Thallassionema nitzschioides	summer
			Asterionella sp.	summer
			Coscinodiscuns marginatus	autumn
			Asterionella japonica	not autumn
Dinophyceae	8	21	*Dinophysis acuta*	all
			Gymnodinium lunula	not winter
			Peridinium ovatum	not winter
			Gonyaulax tamarensis	not winter
			Ceratinum macroceros	not winter
			Ceratinum tripos	not winter
			Dinophysis caudata	spr. & aut.
			Dinophysis norvegica	spr. & sum.
Cyanophyceae	4	4	*Arthospira sp.*	all
			Agmenellum sp.	all
			Oscillatoria sp.	not winter
			Anabaena sp.	sum. & fall
Chrysophyceae	3	3	*Dinobryon balticum*	all
			Distephanus speculum	not winter
			Chrysosphaerella sp.	not winter
Chlorophyceae	2	2	*Scenedesmus acuminatus*	all
			Actinastrum sp.	not winter

Table III Correlation coefficients between chlorophyll, salinity, dissolved oxygen, phosphate, water temperature and phytoplankton numbers in Malpeque Bay, Prince Edward Island.

	Chlorophyll	Plankton Numbers
Chlorophyll	-	.78
Dissolved oxygen	.65	.83
Phosphate	0	0
Salinity	.56	.78
Temperature	.59	.84
Phytoplankton numbers	.78	-

Table IV Seasonal changes in chlorophyll and pheophytin concentrations ($\mu g\ L^{-1}$) in the Bideford estuary, Malpeque Bay, Prince Edward Island.

Date	Station N1	N3	N6
Spring 1973	5	6	3
Summer 1973	6	7	3
Autumn 1973	3	4	3
Winter (Feb.) 1974	3	2	1
Spring 1974	2	1	3
Summer 1974	4	5	5

with the porphyrin levels, and with the water temperature, salinity and dissolved oxygen concentration. There was also some indication that in winter and spring higher levels of the trace metals iron and cadmium were associated with a decrease in phytoplankton numbers.

The high correlation coefficients (Table III) found between phytoplankton numbers and porphyrin levels permits the latter to be used in estimates of population densities. In Table IV changing levels of chlorophyll concentrations with season are given. The results illustrate the validity of this type of estimate of phytoplankton population density, for in all cases a decline in value from summer to winter is observed, followed by a growth in values during the following summer. This conclusion is substantiated by the numbers of cells counted in the water samples collected at stations N1, N3 and N6 over the entire experimental period (Fig 2).

Further analyses of the data were done to evaluate correlation between phytoplankton numbers and the physico-chemical characteristics of the water with respect to season. For the stations in the Bideford estuary in spring the density of the phytoplankton was proved to be realted to the salinity and temperature of the surface water, and its phosphate, silicate and iron content. Similar results were obtained with samples of bottom water taken at the same stations during the summer and winter. It therefore seems probable that nutrient depletion by the phytoplankton population occured during the study period in the N1-N6 stations. The data from the stations largely situated in the open Bay (M1-M16) though less complete suggests that in this environment nutrient depletion is less marked.

Discussion

The experimental data reported in this paper, details of which have been hitherto available only in theses (Sita Devi, 1980), extend the results previously reported by McIver (1972) and by Taylor and his co-workers (1976).

The changing phytoplankton populations of Malpeque Bay illustrate the suggestions of Ryther (1963) that single summer maxima of high production occur in shallow waters. Values found for the chlorophyll levels in water samples during the summer permit a crude estimate of the carbon fixed by the population at this time (Strickland, 1960) to be in the range 5-120 g $m^{-2}\ y^{-1}$. However, the present study does not permit an evaluation of the principal species responsible for this activity. The porphyrin estimations represent the total photosynthetic pigments present in the samples. It is now known (Fogg, 1987) that large numbers of small photosynthetic cyanobacteria are present in marine environments. These organisms would not be retained in our 20 μ collection nets.

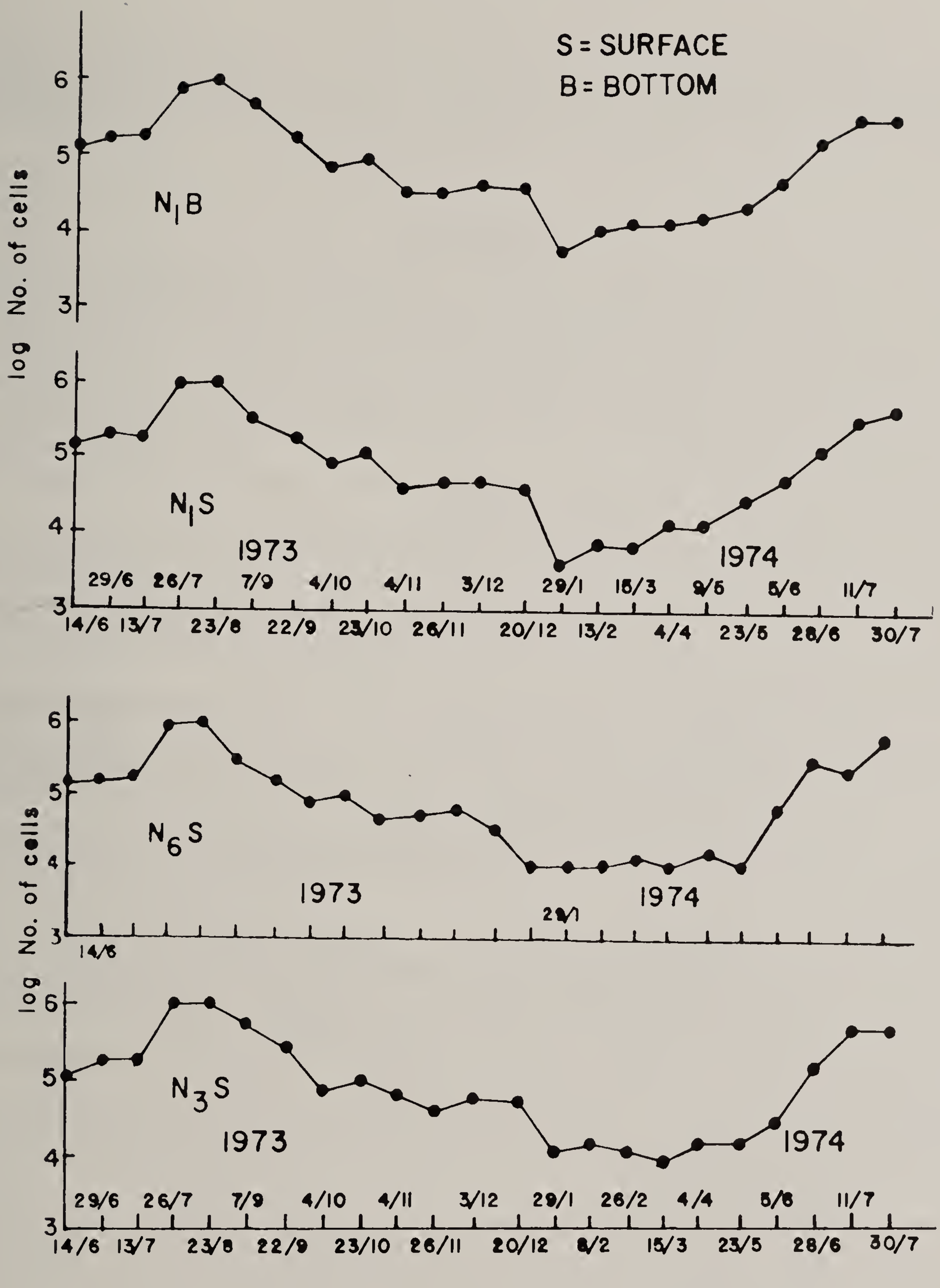

Fig 2 Numbers of phytoplankton collected at sites: N1 (surface (S) and bottom (B) waters; N3 (surface water) and N6 (surface water).

The higher population densities in the esturine stations (N1-N6) than in the more open Bay stations (M1-M16) cannot be solely attributed to higher nutrient levels in the estuary. The equilibrium between supply and removal of nutrients depends on the efficiency of mixing and it may be surmised that the conditions at the M stations were more akin to the steady state thought to be present in the open ocean (Spencer, 1975).

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(Received 28 December 1988.)

APPENDIX I List of Phytoplankton Recorded from Malpeque Bay, Prince Edward Island, Canada.

BACILLARIOPHYCEAE
Order: BACILLARIALES
Sub-Order: COSCINODISCINEAE
Family: COSCINODISCACEAE
Melosira Agardh
M. nummuloides (Dillw.) Agardh
M. juergensii Agardh
Paralia Heiberg
P. sulcata (Ehrenb.) Cleve
Cyclotella (Kützing) de Brébisson
C. striata (Kütz.) Grun.
C. meneghiniana Kütz.
Coscinodiscus Ehrenberg
C. radiatus Ehrenb.
C. concinnus Wm. Sm.
C. centralis Ehrenb.
C. lineatus Ehrenb.
C. marginatus Ehrenb.
C. eccentricus Ehrenb.
Actinocyclus Ehrenberg
A. octanarius Ehrenb.
Thalassiosira Cleve
T. gravida Cleve
T. condensata Cleve
T. decipiens (Grun.) Jörg.
T. nordenskioldii Cleve
Coscinosira Gran
C. polychorda (Gran) Gran
Hyalodiscus Ehrenberg
H. scoticus (Kütz.) Grun.
Skeletonema Greville
S. costatum (Grevi.) Cleve

Family: ACTINODISCACEAE
Actinoptychus Ehrenberg
A. senarius (Ehrenb.) Ehrenb.
Sub-order: AULISCINEAE
Family: AULISCACEAE
Auliscus Ehrenberg
A. sculptus (Wm. Sm.) Ralfs ex Pritch.
A. incertus A.S.
Sub-order: BIDDULPHINEAE
Family: BIDDULPHIAEAE
Biddulphia Gray
B. pulchella Gray
B. aurita (Lyngb.) de Bréb.
B. rhombus (Ehrenb.)
B. mobiliensis (Bail.) ex Van Heurck
B. laevis Ehrenb.
Eucampia Ehrenberg.
E. zodiacus Ehrenb.
Triceratium Ehrenb.
T. alternans Bail.
Isthmia Agardh
I. nervosa Kütz.
Family: CHAETOCERACEAE
Chaetoceros Ehrenberg
C. boreale Bail.
C. atlanticum Cleve
C. convolutum Castr.
C. concavicorne Mangin
C. constrictum Gran
C. didymum Ehrenb.
C. affine Laud.

C. laciniosum Schütt
C. diadema (Ehrenb.) Gran
C. septentrionale Oestrup
C. decipiens Cleve
C. lorenzianum Grun.
C. danicum Cleve
Sub-Order: RHIZOSOLENINEAE
Family: LEPTOCYLINDRAECEAE
Leptocylindrus Cleve
L. danicus Cleve
L. minimus Gran
Guinardia Peragallo
G. flaccida (Castr.) Perag.
Family: RHIZOSOLENIACEAE
Rhizosolenia Brightwell
R. fragilissima Berg.
R. styliformis Brightw.
R. setigera Brightw.
R. alata Brightw.
R. habetata Bail.
Sub-order: FRAGILARINEAE
Family: FRAGILARIACEAE
Fragilaria Lyngbye
F. crotonensis Kitton
F. pinnata Ehrenb.
F. striatula Lyngb.
F. schulzi Brockm.
Raphoneis Ehrenberg
R. amphiceros (Ehrenb.) Ehrenb.
Dimeregramma Ralfs ex Pritchard
D. marinum (Greg.) Ralfs ex Pritch.
D. minor (Greg.) Ralfs ex Pritch.
Asterionella Hassall
A. japonica Cleve & Möller
S. Sp.
Striatella Agardh
S. unipunctata (Lyngb.) Agardh
Synedra Ehrenberg
S. affinis Kütz.
S. pulchella Kütz.
Thalassiothrix Cleve & Grunow
T. longissima Cleve & Grun.
Thalassionema Grunow ex Hustedt
T. nitzschioides Hust.
Plagiogramma Greville
P. staurophorum (Greg.) Heib.
Licmophora Agardh
L. lyngbyei (Kütz.) Grun. ex Van Heurck
L. paradoxa (Lyngb.) Agardh
L. gracillis (Ehrenb.) Grun.
Grammatophora Ehrenberg
G. marina (Lyngb.) Kütz.
G. angulosa Ehrenb.
G. stricta Ehrenb.
Rhabdonema Kütz.
R. minutum Kütz.
Sub-order: ACHNANTHINEAE
Family: ACNANTHACEAE
Achnanthes Bory
A. brevipes Ag.
A. longipes Ag.
Cocconeis Ehrenberg
C. scutellum Ehrenb.
Rhoicosphenia Grunow
R. curvata (Kütz.) Grun.
Sub-order: NAVICULINEAE
Family: NAVICULACEAE
Navicula Bory
N. cryptocephala Kütz.
N. granulata Bail.
N. digito-radiata (Greg.) Ralfs
N. grevilleana Hendey
N. peregrina (Ehrenb.) Kütz.
Stauroneis Ehrenberg
S. salina Wm. Sm.
S. gracilis Ehrenb.
Diploneis Ehrenberg.
D. smithii (de Bréb.) Cleve var. *Smithii*
D. didyma (Ehrenb.) Cleve
D. littoralis (Donk.) Cleve
D. elliptica (Kütz.) Cleve
Caloneis Cleve
C. brevis (Greg.) Cleve
C. westii (Wm. Sm.) Hendey
Pinnularia Ehrenberg
P. quadratarea (Schm.) Cleve
Trachyneis Cleve
T. aspera (Ehrenb.) Cleve
Mastogloia Thwaites in Wm. Smith
M. pumila (Grun.) Cleve
Amphipleura Kützing
A. rutilans (Trent.) Cleve
Brebissonia Grunow
B. boeckii Wm. Sm.
Pleurosigma Wm. Sm.
P. strigosum Wm. Sm.
P. normanii Ralfs
P. decorum Wm. Sm.
Gyrosigma Hassall
G. balticum (Ehrenb.) Cleve
G. wansbeckii (Donk.) Cleve

G. fasciola (Ehrenb.) Cleve
Amphiprora Ehrenberg
A. alata (Ehrenb.) Kütz.
Tropidoneis Cleve
T. vitrea (Wm. Sm.) Cleve
Family: AURICULACEAE
Auricula Castracane
A. Sp.
Family: CYMBELLACEAE
Amphora Ehrenberg
A. robusta Greg.
A. hyalina Kütz.
A. obtusa Greg.
Family: EPITHEMIAECAE
Epithemia de Brébisson
E. turgida (Ehrenb.) Kütz.
E. gibba Kütz.
E. zebra (Ehrenb.) Kütz.
Rhopalodia O. Müller
R. gibberrula var. *producta* (Grun. Müll.
Family: BACILLARIACEAE
Bacillaria Gmelin
B. paxillifer (Müll.) Hendey
Nitzschia Hassall
N. closterium (Ehrenb.) Wm. Sm.
N. angularis Wm. Sm.
N. seriata Cleve
N. spathulata Wm. Sm.
N. obtusa Wm. Sm.
N. apiculata (Greg.) Grun.
N longissima (de Bréb.) Ralfs ex Pritchard
N. socialis Ralfs var. *socialis*
Sub-Order: SURIRELLINEAE
Family: SURIRELLACEAE
Surirella Turpin
S. ovata Kütz.
S. fastuosa (Ehrenb.) Kütz.
Campylodiscus Ehrenb.
C. Sp.
Class: DINOPHYCEAE
Order: DINOPHYSIALES
Family: DINOPHYSIACEAE
Dinophysis Ehrenberg
D. acuta Ehrenb.
D. caudata Savilli-Kent
D. norvegica Clap. et Lachm.
D. Sp.
Order: GYMNODINIALES
Family: GYMNODINIACEAE
Amphidinium Claparédé et Lachmann
A. ovum C. Herdm.
A. Sp.
Gymnodinium Stein
G. lunula Schütt
G. punctatum Pouchet
Gyrodinium Kofoid et Swezy
G. spirale (Bergh.) Kof. et Swezy
G. Sp.
Family: PERIDINIACEAE
Peridinium Ehrenberg
P. ovatum (Pouchet) Schütt
P. Sp.
Family: GONYAULACACEAE
Gonyaulax Diesing
G. tamarensis Lebour
G. catenata (Levender) Kof.
Family: CERATIACEAE
Ceratium Schrank
C. arcticum (Ehrenb.) Cleve
C. furca (Ehrenb.) Clap. et Lachm.
C. fusus (Ehrenb.) Dujard
C. longipes (Bail.) Gran
C. macroceros (Ehrenb.) Vanhöffen
C. tripos (O. F. Müll.) Nitzsch.
Family: OXYTOXACEAE
Oxytoxum Stein
O. Sp.

CHLOROPHYTA
Order: CHLOROCOCCALES
Family: SCENEDESMACCEAE
Scenedesmus Meyen
S. acuminatus (Lagerh.) Chodat
Actinastrum Lagerheim
A. Sp.

CYANOPHYTA
Order: NOSTOCALES
Family: OSCILLATORIACEAE
Oscillatoriaceae Vaucher ex Gomont
O. Sp.
Arthospira Strizenberger ex Gomont
A. Sp.
Family: NOSTOCACEAE
Anabaena Bory
A. Sp.
Order: CHROOCOCCALES
Family: CHROOCOCCACEAE
Agmenellum Bregisson
A. Sp.

CHRYSOPHYTA
Order: OCHROMONADALES
Family: DINOBRYACEAE
Dinobryon Ehrenberg
D. Balticum (Schütt) Lemm.
Family: SYNURACEAE
Chrysosphaerella Lauterborn
C. Sp.
Order: DICTYOCHALES
Family: DICTYOCHACEAE
Distephanus Stöhr
D. speculum (Ehrenb.) Haeckel

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ANTIBIOTIC ACTIVITY OF THE MARINE FUNGUS LEPTOSPHAERIA ORAEMARIS[1]

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Two new pigments, obioninene and oraemarin from the lignicolous marine fungus *Leptosphaeria oraemaris* are described. The effect of salinity on the production of antibiotic compounds from this organism was investigated. Activity of extracts in the HeLa cell bioassay was greatest for fermentations of lower salinity. Toxicity of extracts to brine shrimp larvae was greatest at 14, 18and $22^{0}/_{00}$. In contrast, activity against a marine bacterium was greatest in extracts from fermentations from higher salinities ($>26^{0}/_{00}$). The results are discussed in relation to the ecology of *L. oraemaris*.

Deux nouveaux pigments, l'obioninene et l'oraemarin, obtenus du fungus marin lignicole *Leptosphaeria oraemaris*, sont décrits. L'effet de la salinité sur la production de composés antibiotiques par cet organisme a été étudié. L'activité biologique, telle que mesurée par la toxicité envers les cellules HeLa, fut inversement proportionnelle à la salinité de la fermentation. La toxicité des extraits envers les larves de crevettes de mer fut plus élevée à 14, 18 et $22^{0}/_{00}$. Par contre, l'activité contre une bactérie fut plus élevée pour les fermentations à haute salinité ($>26^{0}/_{00}$). Les résultats sont discutés en de l'écologie de *L. oraemaris*.

Introduction

Miller & Whitney (1981) recorded lignicolous marine fungi from the three classes of wood in the sea: drift wood, intertidal wood and sterile panels of wood used as and showed that fungal diversity decreases among these three, a fact often reported others (Kohlmeyer & Kohlmeyer 1979). Close examination of such wood reveals signs of interference competition between some of the marine fungi present, something that appears also to occur under controlled conditions (Miller *et al.* 1985, Strongman et al. 1987).

Interference competition in lignicolous fungi can be mediated by the production of antibiotic metabolites including volatiles and possibly by the production of compounds that sequester nutrients (Wicklow 1981, Bruce *et al.* 1984). The substrate on which aquatic lignicolous fungi can be found, i.e. wet or waterlogged wood, would appear to limit interference competition. Compounds that are produced might be expected to leach away before having any effect. None the less, the production of

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antimicrobial compounds by both freshwater (Fisher & Anson 1983; Shearer & Zare-Maivan 1988) and marine (Miller *et al.* 1985, Strongman *et al.* 1987) lignicolous fungi has been reported. These authors considered that antibiosis detected *in vitro* played a role in determining the population structure of the fungi involved despite the obvious objections discussed by Shearer & Zare-Maivan (1988). Although most of the above workers were concerned with antifungal activity, marine fungi produce compounds that are antibacterial and toxic to wood-associated fauna (Miller 1986).

Marine fungi growing on intertidal wood are exposed to variations in moisture, temperature and, depending on rainfall and land run-off, salinity. There are average differences between sites, and often profound differences on a daily basis. This is particularly true for sites in eastern Canada such as the Bay of Fundy and the shore of Prince Edward Island, locations with extreme tidal range and ice in the winter, respectively (Miller & Whitney 1981, Strongman *et al.* 1985). The optimum salinity for growth of lignicolous marine fungi is approximately $28^0/_{00}$ at 25°C. Regulation of osmoticum in higher marine fungi is thought to involve the production of sugar alcohols, particularly glycerol (Jennings 1983, Wethered *et al.* 1985). In filamentous fungi, the production of antibiotics involves the diversion of various primary metabolites to secondary metabolism (Bu'Lock 1975). The effects of salinity stress could be postulated therefore to include effects on secondary metabolism.

This strain of *Leptosphaeria oraemaris* Linder has been reported to produce the antifungal sesquiterpene diol culmorin (Strongman *et al.* 1987). This species has also been reported to produce the aminohexose leptosphaerin, and other metabolites (Miller *et al.* 1984; Schiehser *et al.* 1986; White *et al.* 1989). The purpose of this report is to describe experiments characterizing a new metabolite from *L. oraemaris* and to demonstrate the effect of salinity on the production of crude extracts toxic in various bioassays.

Materials and Methods

Fermentations. Leptosphaeria oraemaris (Strongman *et al.* 1987) was maintained on seawater agar (1 g glucose, 1 g yeast extract, 1 g peptone (all Difco) per L seawater $28^0/_{00}$; Johnston & Sparrow 1961). A seawater agar slant of the culture was macerated in 50 mL sterile artificial seawater (without N or P; Churchland & McLaren 1976). The resulting suspension was used to inoculate (5% v/v) six 250 mL Erlenmeyer flasks containing 50 mL of the following medium: 2 g NH_4Cl, 0.2 g $FeSO_4$, 2 g KH_2PO_4, 2 g peptone, 2 g yeast extract, 2 g malt extract and 20 g glucose per L of $28^0/_{00}$ artificial seawater. The flasks were put on a rotary shaker at 220 rpm (3.81 cm throw in a horizontal plane) at 25°C for three days. The resulting mycelium was macerated, washed in sterile artificial seawater ($28^0/_{00}$) by centrifugation and resuspended to deliver 2 mg dry weight in 1 mL sterile artificial seawater (Miller *et al.* 1984). This was used to inoculate (5% v/v) each of six 250 mL Erlenmeyer flasks containing 50mL of 10 g glucose, 1 g yeast extract and 1 g peptone per L artificial seawater at salinities of 14, 18, 22, 26, 30 or $34^0/_{00}$, respectively. These were incubated at 25°C for 28 d.

At the end of the incubation period, cultures were filtered through Whatman filter paper (#1), washed and dry weights determined. The mycelia from each salinity were pooled and macerated in 100 mL methyl alcohol, the solution was filtered, taken to dryness and weighed. The extract was then subjected to vacuum liquid chromatography to separate the material into 5 fractions of increasing polarity as follows. Silica gel 60 was put in a 30 mL sintered glass funnel and the extract dissolved in chloroform was layered on the silica. This was eluted with 100 mL aliquots of chloroform/methanol: fraction 1 (100/0), 2 (95/5), 3 (90/10), 4 (85/15), 5 (80/20); fraction 1 was discarded and the rest were dried. These fractions are, henceforth, described as e.g. 14-2, 26-5, the first two digits referring to salinity, the last to fraction number.

Bioassays. The above fractions were tested in three bioassays involving brine shrimp, HeLa cells and a gram-negative marine bacterium freshly isolated on seawater agar from *Fucus vesiculosus* L. obtained from McLaren's Beach, New Brunswick. (Miller & Whitney 1981). Brine shrimp eggs were obtained from a tropical fish store and 20 mg added to artificial seawater ($28^{0}/_{00}$) containing 6 g/L glycine (100 mL in a 250 mL Erlenmeyer flask). These were incubated in the light with sterile air bubbled into the seawater for 24 h at 30°C. Extracts (2-5) dissolved in ethyl alcohol and added to sterile 24-well plates in triplicate at 1, 10, 100 and 1000 μg plus solvent controls. The solvent was allowed to dry in a laminar flow hood and 1 mL of artificial seawater/glycine solution with 25 live brine shrimp was added to each well. The plates were sealed with Parafilm and put in an incubator at 30°C in the light. The wells were inspected each day for three days and those containing $>50\%$ dead brine shrimp were recorded as toxic. Control wells contained $>95\%$ living brine shrimp after three days under these conditions.

The bioassay involving the *Fucus* bacterium was done by the paper disc method. (Vincent & Vincent 1944). A lawn of the marine bacterium was prepared on seawater agar and the agar surface allowed to dry. Antibiotic assay discs (10 mm) were sterilized, 100 μg of each extract in ethanol put on two discs and the solvent was allowed to evaporate under sterile conditions before placement on the agar. After 1 and 2 days, the presence of a zone of inhibition around each disc was recorded (as + or -).

HeLa cells were cultured in Minimal Essential Medium (Gibco #320-1095) plus 5% heat inactivated fetal bovine serum, 14.7 mg per 100 mL glutamic acid and 100 μg/mL gentamycin. Aliquots (1 ml at 10^5 cells/mL) were dispensed into a 24 well plate and allowed to grow for 24 h at 37°C in 4% CO_2. A 2 mg/mL ethyl alcohol solution of each extract was prepared and 1 μL added to each of the three wells (i.e. 2 μg/mL medium). Ethyl alcohol controls and a deoxynivalenol positive control (1 μg/ml) were included in each assay. The cells were examined at 24 and 48 h and extracts that resulted in the death of all or most of the cells were recorded as toxic. Each extract was assayed in two experiments on different days. Results from the bioassays indicated the presence of an active compound(s) in the $18^{0}/_{00}$ fermentation.

Characterization of pigments. ^{1}H and ^{13}C spectra were recorded at 500 MHz and 125 MHz, respectively on a Bruker AM-500 spectrometer or at 250 MHz and 62.9 MHz on a Bruker AM-250 spectrometer. Chemical shifts are referenced to residual $CHCl_3$ at 7.24 ppm for ^{1}H spectra and C^2HCl_3 at 77.0 ppm for ^{13}C spectra and reported relative to tetramethylsilane. Mass spectra were obtained on either a Finnigan MAT 312 mass spectrometer or Finnigan MAT 4500 GC/ms system. Accurate mass measurements were determined by peak matching with an ion in the spectrum of perfluorokerosene.

Isolation of pigments. The mycelium (21 g) from 3 L of culture broth grown at $18^{0}/_{00}$ salinity was collected by filtration. It was dried in air, macerated with ethyl acetate (6 x 200 mL) and the combined ethyl acetate extracts evaporated to give a brown oil (792 mg) which was applied to silica gel and the column eluted with ethyl acetate-hexane (1:1) to provide a red pigment (413 mg). Further chromatography on Sephadex LH-20 using dicholoromethane-hexane (1:1) as the eluant, followed by recrystallization from ethyl acetate-hexane (1:1) gave the naphthoquinone (Fig 2), m. p. 160-162°(dec.), $[\alpha]^{24}_{D}=+52°$ (c: 0.01, $CHCl_3$), λ_{max} (MeOH) 242, 311, 382 nm (ε 13900, 10200, 10700 dm^3 mol^{-1} cm^{-1}, λ_{max} (MeOH + NaOH) 280, 402, 530 nm (ε 10500, 5100, 720 dm^{3}mol^{-1} cm^{-1}), IR ($CHCl_3$) 3090, 2400, 1510, 1425, 1210, 930 cm^{-1}, m/z 354.1494 (100; $C_{21}H_{22}O_5$ requires 354.1467), 339, 325, 307, 297, 285, 269, 257, 214, 149, 109, n.m.r. see Table II. The quinoxaline derivative was prepared in 37% yield by treatment of the metabolite (20 mg) with 1,2-diaminobenzene (40 mg) in ethyl alcohol (17 mL). The reaction was heated under reflux for 15 minutes, cooled and the precipitated quinoxaline (m. p. 233-235°, $[\alpha]^{24}_{D}=+78°$ (c: 0.08, $CHCl_3$), m/z 426 (100), n.m.r. see Table I) recrystallized from diethyl ether.

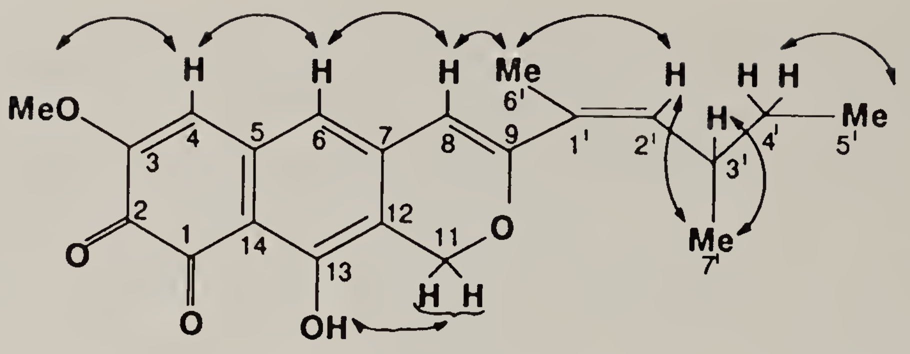

Fig 2 Structure of obioninene; arrows indicate demonstrated nuclear Overhauser effects.

Table I Nuclear magnetic resonance data (^1H & ^{13}C) for obioninene and its quinoxaline derivative. The numbers in column 1 are locants (Fig 2). Coupling constants (*J*) are given in parentheses in hertz (Hz). Chemical shifts are given in parts per million of field strength downfield from the signal of tetramethylsilane.

	Obioninene		Quinoxaline	
	^1H	^{13}C	^1H	^{13}C
1	-	178.8		144.9
2	-	177.0		140.7
3	-	151.7		151.6
4	6.25, s*, 1H	113.9	6.92, s, 1H	108.4
5	-	134.8		134.1
6	6.38, s, 1H	118.0	7.06, s, 1H	113.1
7	-	144.4		136.4
8	5.83, s, 1H	100.4	6.01, s, 1H	100.6
9	-	161.9		157.9
11	5.16, d (13.9 Hz), 1H 5.12, d (13.9 Hz), 1H	62.7	5.47, d (13.2 Hz), 1H 5.44, d (13.2 Hz), 1H	63.7
12		112.6		112.2
13	-	162.1		155.0
14	-	111.1		109.1
1′	-	126.5		126.9
2′	6.22, dq (10.6, 0.9 Hz), 1H	141.2	6.14, dq (9.8, 1.3 Hz), 1H	137.6
3′	2.45, m, 1H	34.9	2.46, m, 1H	34.7
4′	1.42, m, 1H; 1.34, m, 1H	30.0	1.43, m, 1H; 1.36, m, 1H	30.3
5′	0.85, t, (7.3 Hz), 3H	12.0	0.88, t (7.4 Hz), 3H	12.0
6′	1.85, d (0.9 Hz), 3H	12.9	1.91, d (1.4 Hz), 3H	13.0
7′	1.00, d (6.5 Hz), 3H	20.2	1.02, d (6.6 Hz), 3H	20.4
OCH_3	3.80, s, 3H	55.7	4.17, s, 3H	56.3
OH	12.32, s, 1H		14.43, s, 1H	
				138.1
			8.15, d (8.2 Hz), 1H	127.1
			7.84, m	129.5
			7.84, m	130.0
			8.40, d (8.3 Hz), 1H	131.0
				138.9

* Abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet

A second red pigment was obtained from the combined fractions 14-4, 14-5, 18-4, and 18-5. It was purified by flash chromatography on silica gel (230-400 mesh). The column was developed with ethyl acetate-hexane (1:9, 15 mL) and the pigment (3 mg, m/z 366 (100), 351 (53), 337 (58), 322 (23), 311 (8), 284 (19), 279 (20); δ_H (C^2HCl_3) 12.69 (H), 9.38 (H, *J* 0.9 Hz), 8.09 (H, *J* 0.9 Hz), 7.44 (H), 6.54 (H, *J* 9.8 Hz, *J* 1.3 Hz), 4.66 (2H), 4.03 (3H), 2.60 (H, m), 2.18 (3H, d, J 1.2 Hz), 1.45 (2H, m), 1.07 (3H, J 6.7 Hz), 0.90 (3H, t, J 7.5 Hz), δ_c (C^2HCl_3) 148.4, 142.9, 114.8, 104.2, 56.2, 35.4, 30.1, 20.1, 14.4, 12.0) eluted with ethyl acetate-hexane (1:4, 20 mL).

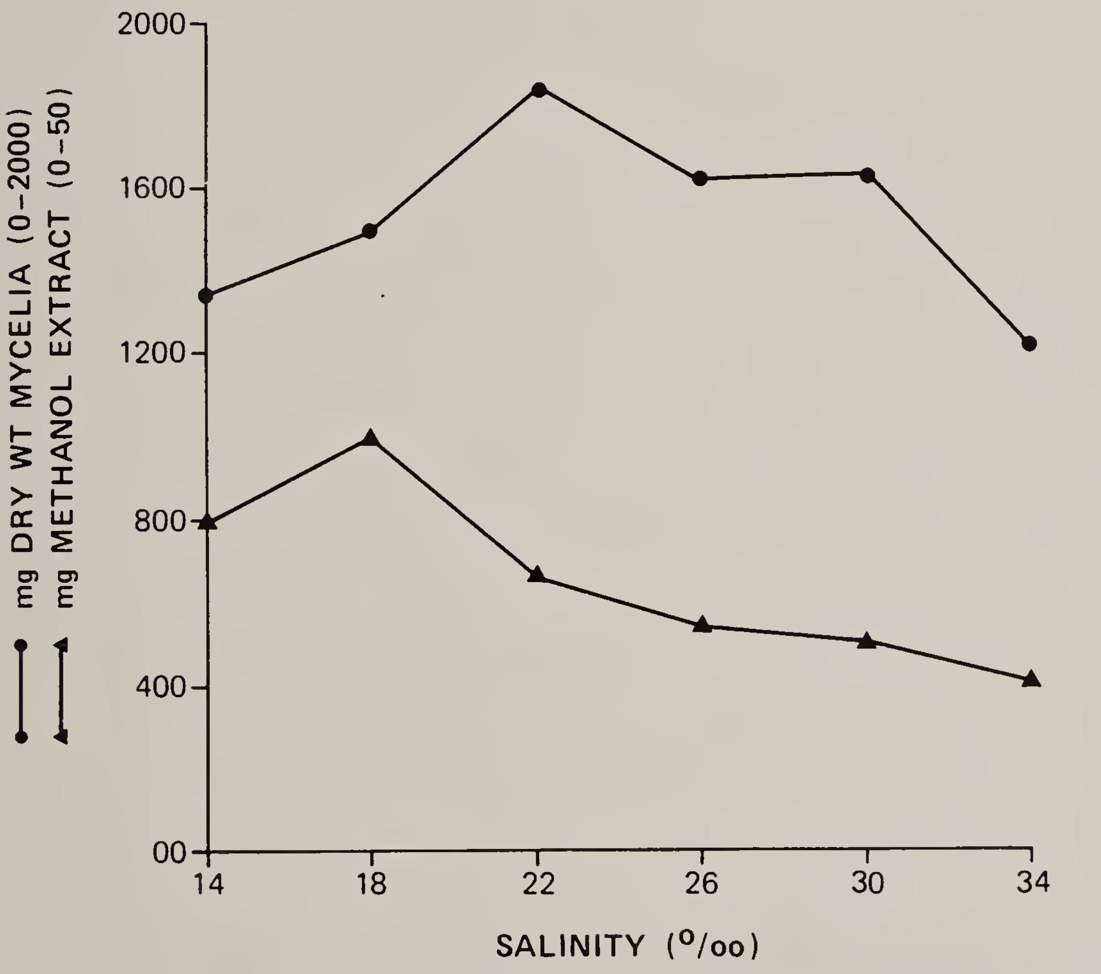

Fig 1 Mycelial dry weight and weight of methyl alcohol extract of cultures of *L. oraemaris* grown at different salinities for 28 days at 25°C. Standard deviations of mycelial and extract weights at each point varied from 5-10% of the mean (n=6).

Results and Discussion

Effects of salinity on antibiotic production

Growth of *Leptosphaeria oraemaris* at different salinities is shown in Fig 1. Maximum dry weight production occurred at ca. 22^{0}/oo at 25°C for this strain. The ratio of the weight of the methanol extract to the mycelia peaked at ca. 18^{0}/oo. Production of red pigment was also maximum at 18^{0}/oo salinity by a factor of two or more compared to that at 34^{0}/oo.

The results of the various bioassays are summarized on Table II. A remarkable pattern of biological activity emerged. The salinities at which the greatest activity occurred for each bioassay were different. HeLa cell toxicity of the fractions decreased as salinity was changed from 14 to 34^{0}/oo. Toxicity to brine shrimp occurred in fractions from fermentations at 14, 18 and 22^{0}/oo, with the maximum occurring at 18^{0}/oo. In contrast, the fractions active against the *Fucus* bacterium came from the

Table II Bioassays of fermentation extracts

Fraction	Brine shrimp larvae[1] (μg/mL)				HeLa cell[2] (2 μg/mL)	Marine bacterium[3] 100 (μg/disc)
$^0/_{00}$-#	1000	100	10	1		
14-2	+	-	-	-	+++++	+
14-3	-	-	-	-	-	-
14-4	-	-	-	-	+	-
14-5	-	-	-	-	+++	-
18-2	-	-	-	-	-	+
18-3	-	-	-	-	+	-
18-4	+	+	-	-	+++++	-
18-5	+	+	+	-	+	-
22-2	+	-	-	-	+++	-
22-3	+	-	-	-	-	-
22-4	+	-	-	-	-	+
22-5	+	-	-	-	-	-
26-2	-	-	-	-	-	+
26-3	-	-	-	-	-	+
26-4	-	-	-	-	-	+
26-5	-	-	-	-	-	+
30-2	-	-	-	-	+	+
30-3	-	-	-	-	++	-
30-4	-	-	-	-	-	+
30-5	-	-	-	-	-	+
34-2	-	-	-	-	++	-
34-3	-	-	-	-	-	+
34-4	-	-	-	-	-	+
34-5	-	-	-	-	-	+
culmorin	-	-	-	-	-	+

[1] 25 live brine shrimp added to each well of a 24 well plate containing artificial seawater/glycine medium. The plates were inspected each day for 3 days and those wells containing >50% dead brine shrimp were recorded as toxic (+). Control wells and ethanol controls contained >95% living brine shrimp).

[2] HeLa cell assay: - = no damage, +++++ damage equal to 1 μg/mL deoxynivalenol.

[3] Definite zone of inhibition observed = +.

fermentations in the higher salinities. Although the strain of *L. oraemaris* has been reported to produce culmorin, this compound was not produced under these fermentation conditions. Culmorin was not bioactive in the brine shrimp and Hela cell assays at the concentrations tested (Table II).

Bolton *et al.* (1988) reported the effect of salinity on antibiotic production in 17 species of lignicolous marine fungi. Methanol extracts of the mycelia grown at different salinities were tested for activity against a number of medically important bacteria, *Saccharomyces* and *Mucor.* For most of the fungi tested, the use of full strength seawater resulted in decreased antibiotic production. In the case of *Aniptodera marina* Shearer and *Lulworthia* sp., antifungal activity was only expressed or increased markedly, respectively, in the fermentation with seawater. An unidentified *Leptosphaeria* species produced similar activity against *Bacillus subtilis* (Ehrenberg) Cohn regardless of salinity. In general, these authors reported that salinity affected the qualitative and quantitative distribution of antibiotic production in the bioassays used. This is consistent with the present data.

L. oraemaris is distributed in cool waters on saltmarsh plants, drift and intertidal wood and test panels. On the basis of distribution and habitat analysis, this species has been further characterized as eryhalothermic or found in areas with salinity change >5% (Booth & Kenkel 1986). This assignment is consistent with reports from Atlantic Canada i.e. the fungus has been reported from the Bay of Fundy and coastal Prince Edward Island but not from homeohaline Halifax or Argentia (Meyers & Reynolds 1960; Miller & Whitney 1981; Strongman *et al.* 1985). The response to salinity with respect to the production of toxic metabolites demonstrated by this fungus accords with the environmental conditions in the preferred habitat. *L. oraemaris* in this habitat will be exposed to a range of salinity and hence the antibiosis observed *in vitro* could be expected to be produced *in vivo*. These data argue that consideration of salinity as a variable is required in studies of the production of metabolites from higher marine fungi. Additionally, *L. oraemaris* produces metabolites which are active against microbial competitors (bacteria and fungi) and fauna that might be expected to consume mycelia (Miller 1986; Strongman *et al.* 1987).

Characterization of pigments. The main pigment obtained, $C_{21}H_{22}O_5$ was shown to be an orthoquinone by its reaction with 1,2-diaminobenzene to give a quinoxaline whose molecular weight was 426. The absorption of the quinone at 482 nm, shifted to 530 nm in alkaline solution, indicated the presence of a phenol (δ_H 12.32) and a fourth oxygen atom was accounted for by the methoxyl resonance at 3.80 p.p.m. The nature of the asymmetry in the molecule ($[\alpha]_D$+58°) was indicated by the coupling of the proton at 2.45 p.p.m. with the methyl group at 1 p.p.m. and with a methylene group (δ_H 1.34, 1.42) which in turn was coupled to a second methyl group at 0.85 p.p.m. The proton at 2.45 p.p.m. was also coupled to an olefinic proton (δ_H 6.22) which was allylically coupled to a third methyl group (^4J 0.9 Hz). These data, fully consistent with ^{13}C n.m.r. data indicate the presence of a 1,3-dimethylpent-1-enyl moiety analogous to the 1,3-dimethylpentane side chain thought to be present in obionin A (Poch & Gloer 1989). Evidence that the orientation of the methoxyl group was the same as in obionin A was obtained by nuclear Overhauser effect experiments; positive effects are indicated in Fig 2 by arrows. Thus the pigment is probably an unsaturated obionin A and the formula in Fig 2 is consistent with the data obtained.

A small quantity of a second, unstable red pigment, named oraemarin, was isolated from fractions 4 and 5 of cultures grown in $14^{0}/_{00}$ and $18^{0}/_{00}$ salinity. Some of its physical properties are given above, and they suggest that it is related to obioninene.

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LOCATION OF FORAGING ACTIVITY BY BLACK DUCK (ANAS RUBRIPES) BROODS IN A NOVA SCOTIA ESTURINE MARSH

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Use of habitat for foraging by black duck (*Anas rubripes*) broods was investigated in a Nova Scotia estuarine marsh during the 1988 brood rearing season. Some data for other years are reported. Algal and flowering plant species, water chemistry and several physical factors such as depth of water and degree of exposure to wind/tide, were recorded for each of 51 sampling plots (15 x 15 m). Ducklings were nonrandomly distributed in the marsh. Principal component analysis (PCA) of utilized sites alone, indicated a relationship between duckling distribution and the presence of floating algal mats, which included species of *Rhizoclonium, Cladophora*, and *Ectocarpaceae*. Prior to the appearance of algal mats in late June, ducklings were not associated with specific vegetation types, but were associated with sheltered sites.

On a éxaminé l'usage de l'habitat en but de fourrager par des couvées du canard noir (*Anas rubripes*) dans un marais de la Nouvelle-Ecosse en 1988 durant la saison de l'élevage des couvées. Des données se rapportant à d'autres années sont aussi presentées. On a rapporté les espèces d'algues et des plantes à fleurs, la chimie de l'eau, et quelques facteurs physiques tels que la creuseur de l'eau, et le taux d'exposition au vent et aux marées pour chacune des 51 parcelles d'échantillonage (15 x 15 m). Les canetons n'étaient pas ditribuées au hasard dans le marais. Une analyse des composants principaux des lieux utilisés a démontré un rapport entre la distribution des canetons et la présence de tapis d'algues flottantes comprenant des espèces de *Rhizoclonium*, de *Cladophora*, et des *Ectocarpaceae*. Avant l'apparition des tapis d'algues (vers la fin de juin), les canetons n'étaient pas associées avec des types spécifiques de végétation, mais démontraient un rapport avec des sites abrités.

Introduction

Many workers have studied habitat use by ducks, but there are few studies on brood use of habitat for foraging (Bengtson, 1972; Sugden, 1973; Ringelman and Flake, 1980). Reinecke (1979); Ringelman and Longcore (1982) and Hickey and Titman (1983) investigated aspects of the ecology of black duck (*Anas rubripes*) broods, but the study by Courcelles and Bédard (1979) is the only one to report details of use of a fresh water marsh by broods of dabbling ducks (Anatini), including the black duck. They showed that broods preferred habitat characterized by specific aquatic plants and correlated this with physical and chemical features of the habitat.

MacLean (1988) studied black duck brood mortality in a Nova Scotia estuarine habitat, but no attempt was made to correlate habitat use with plant community types. Our study was done on the same marsh and the objectives were to determine the location of foraging activity by black duck broods throughout the brood rearing period, to test the hypothesis that broods were randomly distributed within a zone of frequent use, and to attempt to link foraging activitity to specific patterns of vegetation.

Materials and Methods

The study area was a heterogeneous 1.5 km^2 tidal marsh at the N.W. end of a 10 x 2 km estuary that drains a 750 km^2 watershed into St. Georges Bay in the outer St.

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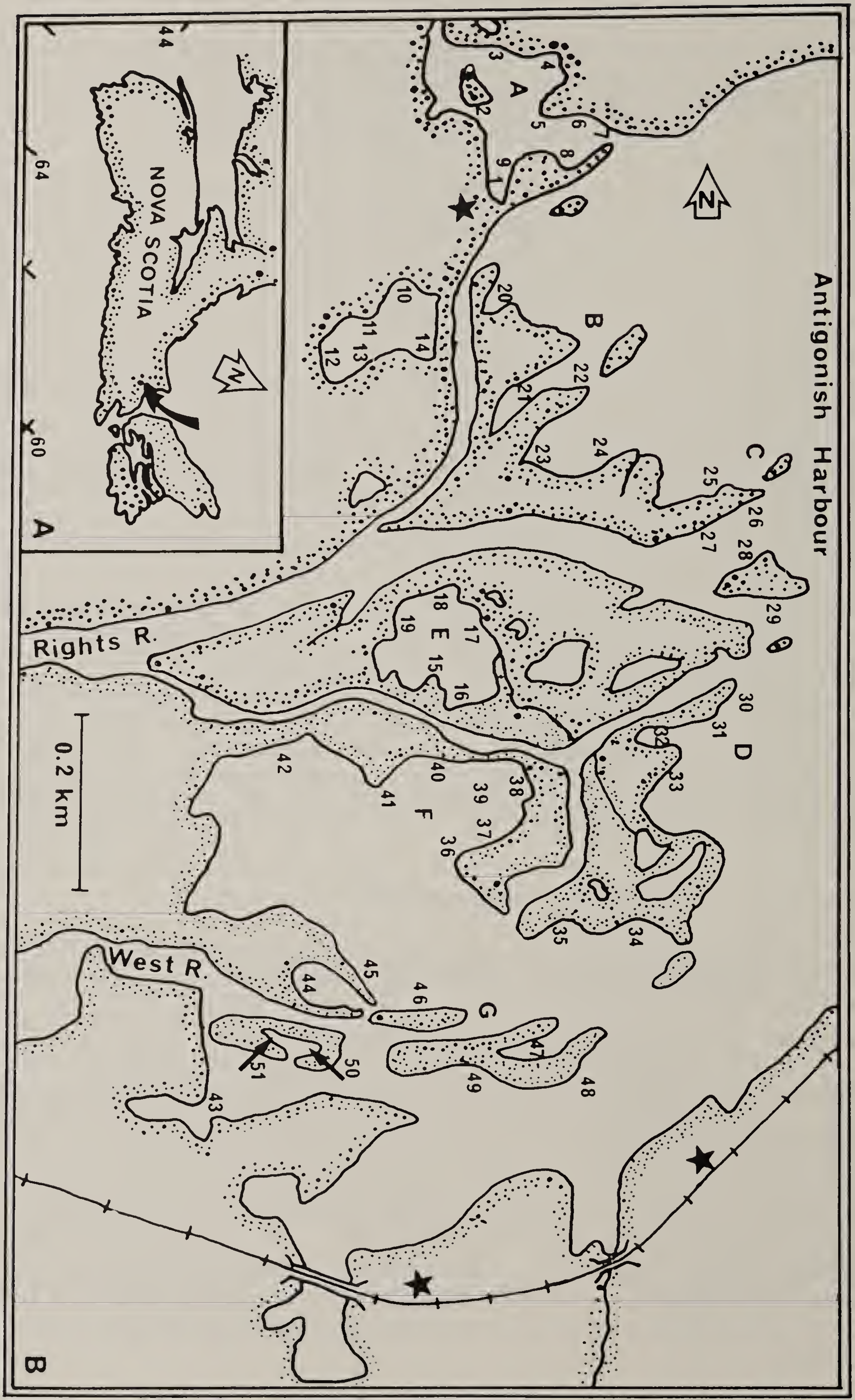

Fig 1 A) Map of Nova Scotia with arrow indicating general location of Antigonish Harbour. B) Detail of Spartina marsh at head of Antigonish Harbour showing locations of sampling sites. Note groupings of sites A-G. Stars indicate observation points.

Lawrence River estuary near Antigonish, Nova Scotia (Fig 1). Several topographically distinct areas of the marsh can be identified according to water depth, extent of tidal influence, sediment composition, degree of exposure to wind/wave action and availability of cover that provided ducklings protection from predators and/or severe weather.

Except for two river channels and associated small backwaters and gullies, the upper part of the marsh was a continuous, dense stand of largely terrestrial vegetation (trees, shrubs, herbaceous plants and grasses). This gave way to an inter-tidal zone of predominantly emergent plants dominated by *Spartina alternaflora,* and associated *S. patens* and *S. pectinata, Juncus* spp. and *Carex paleacea.* Much of this zone held permanent water, or experienced periodic tidal flooding. Dense stands of submergent vegetation, predominantly *Potamogeton* spp., and an associated complex of algae *Cladophora* spp., *Enteromorpha* spp., *Rhizoclonium riparium* and *Spirogyra* sp.) characterized the open water for 50-100 m beyond the highly indented marsh edge, and in the coves, ponds, inlets and 5 permanently water-filled tidal pools at the periphery (Fig 1). Algae formed floating mats over the pond weeds after mid-June. Fig 1 shows the indented ($\simeq$15.25 km) nature of the interface between water/vegetation in the outer $\simeq$ 70 ha of the marsh where most brood activity occurred.

Each year, from 1972 - 1988, the marsh was used for brood rearing from late May until late August by several females ($\bar{x}$ = 11, range 8-16). More information on this population of black ducks can be found in Seymour and Titman (1978, 1979) and Seymour (1984).

Field Studies Regular monitoring of the marsh provided a profile of seasonal changes in vegetation. Prior to the appearance of broods, fifty-one 5 m^2 sites were selected and delineated (Fig 1). Sites were either over open water, or at the interface between emergent vegetation and water. Samples of algae and flowering plants, as well as water samples for chemical analysis, were taken at each site during 3 periods (7-14 June, 13-21 July, 15-25 Aug.). Random samples of algae were identified for each plot. All submergent and emergent vegetation was removed from 3 randomly selected 0.25^2 quadrats within 3 m - long strips at the interface ($\simeq$1.5 m of water/1.5 m of marsh) of each plot. Vegetation was taken to the laboratory where wet biomass, and approximate density of each species were determined for each quadrat.

The distribution of 15 broods (70 ducklings) was determined during road transects that allowed complete surveillance of the part of the marsh used for foraging by broods. Transect observations of broods were related to the plots used to collect environmental data. Only location of foraging activity was examined and potential resting areas were not observed. Ninety-three transects (160 h observation) were made over the entire brood-rearing period. Most transects were conducted during the daylight hours of early morning or evening when broods on this marsh forage most actively (Seymour unpublished observation). The number of ducklings present, and whether they were foraging or resting were recorded during each transect. It was determined that birds were foraging by observing their behavior for periods of 10-45 min. Frequently several broods could be simultaneously observed. No attempt was made to determine either food selection, or availability of food. A subjective value, where 5 indicated high exposure on a scale of 1-5, was used to estimate wind/wave action at each site. Additional data presented in the results section of this paper were collected in 1983, 1986, 1987 during studies and of breeding black duck pairs. The same transects and schedule of monitoring birds used in this current study was used then.

Data Analysis Morisita's index of dispersion (Zar, 1984) was used to test for non-random distribution patterns of broods. The significance of the resulting values was investigated using standard chi-square tests. Principal component analysis (PCA) (Sneath and Sokal, 1973; Clifford and Stephenson, 1975; Seber, 1984) was used to further discern spatial variation among sites. Principal component analysis selects a sequence of (linear) combinations of the (random) variables which explain a large proportion of the total dispersion of the sample covariance matrix. The first principal component has or explains the maximum variance among all possible (linear) combinations of the variables while subsequent principal components have or explain the maximum variance among all possible (linear) combinations of the variables which are uncorrelated with previous principal components. The distance measure used was standard Euclidean metric. PCA was carried out using algorithms in Systat (Wilkinson, 1986). Analysis was done on data sets containing presence or absence information for each of the algal and higher plant species, pH, salinity, temperature, and the subjective shelter values for each sample site. Further analysis utilizing quantitative measures of higher plant density and wet biomass were also carried out.

Results

Although the area of marsh and tidal pools was 150 ha, almost all foraging occurred in the outer 70 ha, as was noted during studies of black duck broods in 1983 (216 h observation of 12 broods), 1986 (309 h observation of 16 broods), and 1987 (301 h observation of 18 broods). However, foraging activity was restricted to a 5 m wide zone at the interface of emergent vegetation and water, except for forays of up to 30 m from the edges, over mats of vegetation in July and August. Indeed, most foraging

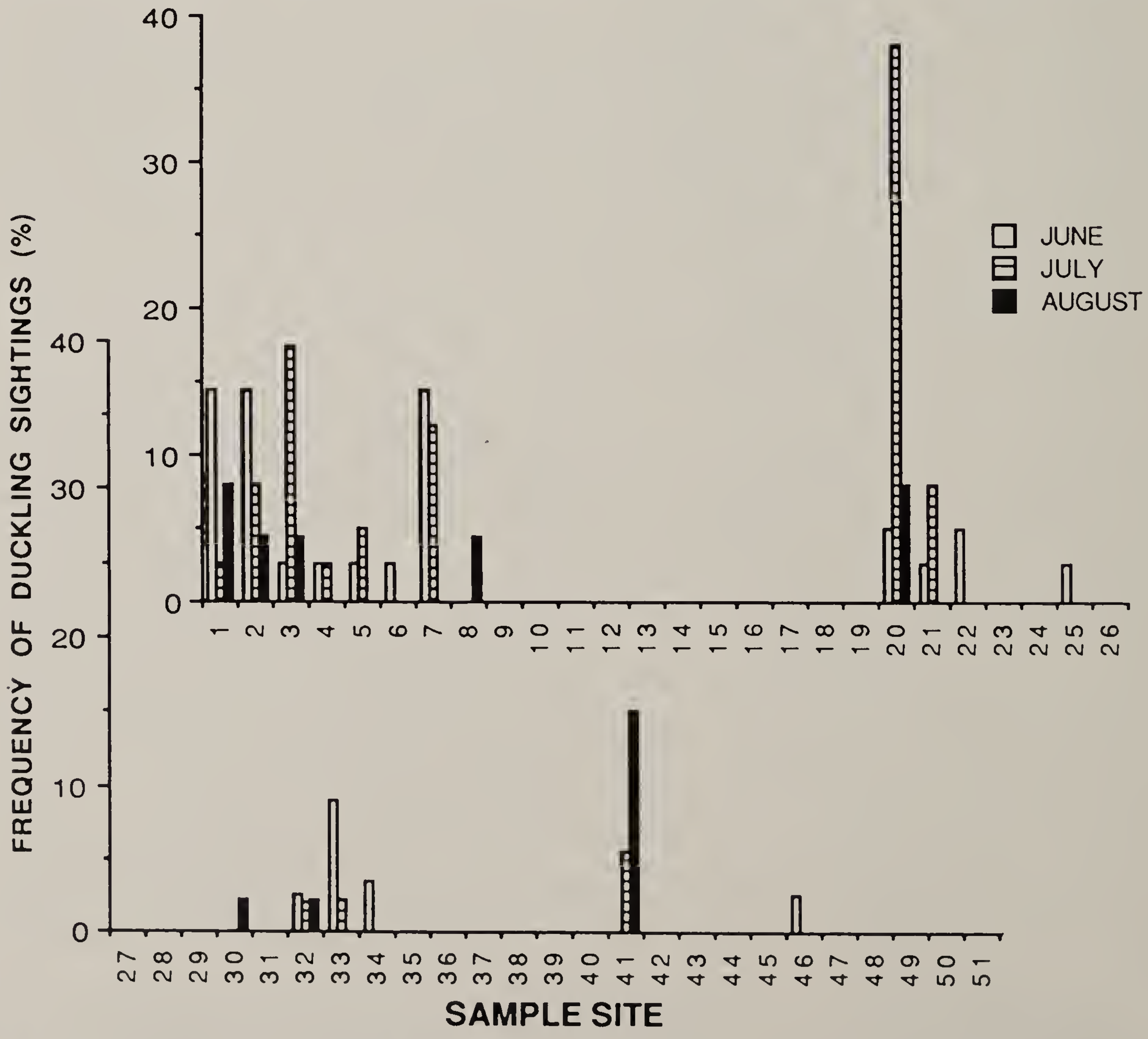

Fig 2 Histogram indicating frequency of brood occurrence at study sites from May to August, 1988 in Spartina marsh at head of Antigonish Harbour.

Table I Rotated factor loadings on axes 1 and 2 for ordination shown in Fig 3. Note: 18 factors loading high on axes 1 and 2 are included.

Species	Factor 1	Factor 2
Variance explained	16.2%	10.8%
Rhizoclonium riparium (Roth) Harvey	0.703	0.002
blue-green algae	0.671	-0.247
Agrostis alba L.	-0.569	-0.402
Ectocarpus sp.	0.549	-0.047
Solidago sempervirens L.	-0.527	0.132
Spartina patens (Ait.) Muhl.	-0.525	-0.422
Cladophora sp.	0.188	0.714
Spirogyra sp.	0.372	0.638
Spartina pectinata Link	-0.016	0.605
salinity	0.033	-0.598
pH	0.103	-0.556
Enteromorpha spp.	0.487	0.538
Scirpus maritimus	-0.458	0.538
shelter	0.416	0.231
Capsosiphon fulvescens (C. Ag.) Setch. & Gard.	0.310	-0.197
Klebsormidium sp.	0.463	0.175
Blidingia spp.	0.372	0.064
Pilayella littoralis (L.) Kjellm.	0.353	-0.077

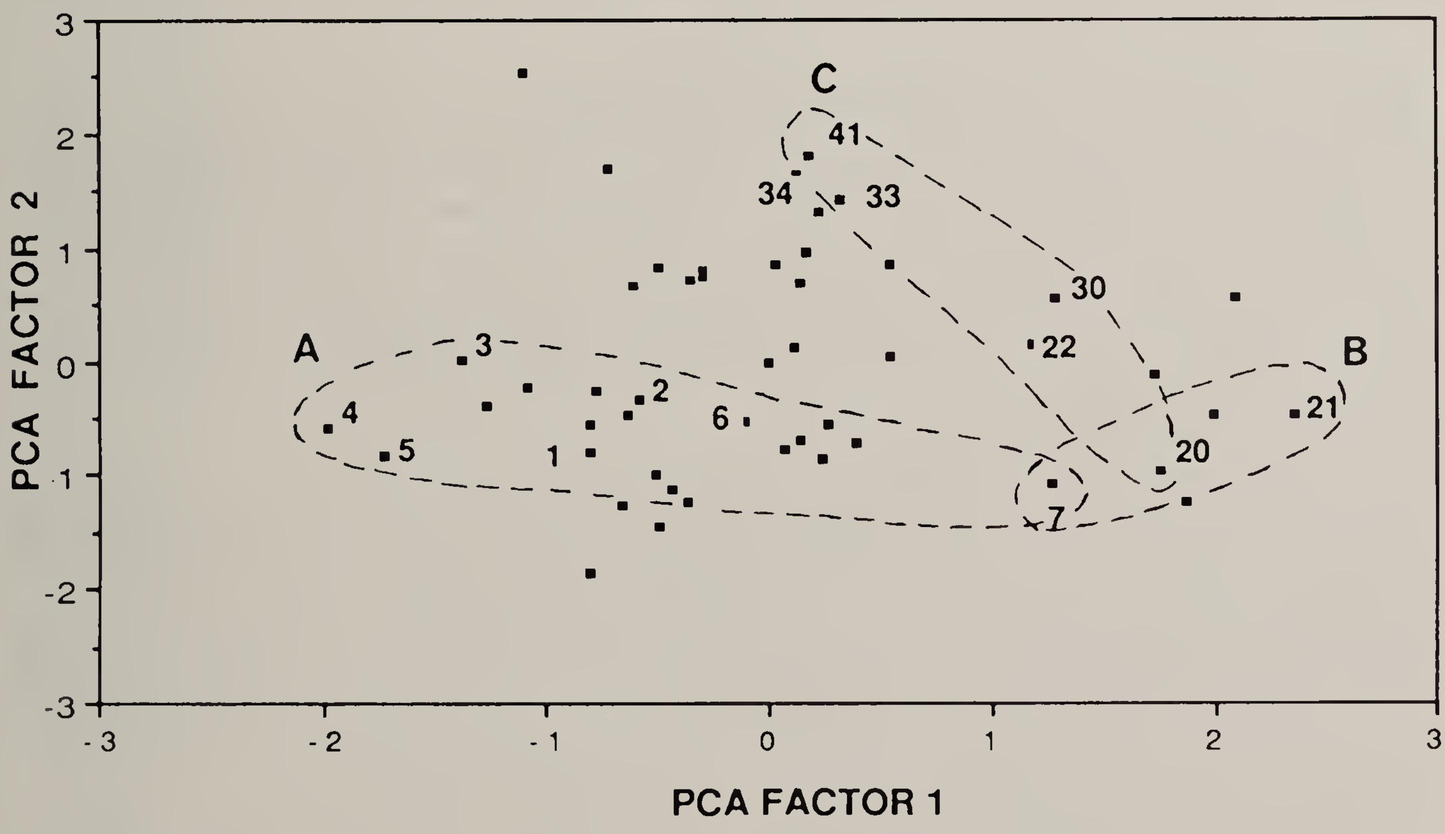

Fig 3 Ordination of sampling sites based on principal component analysis using physical environmental data, and presence/absence of flowering plants and algae. Numbered sites indicate those most highly utilized (*cf.* Fig 1). A indicates highly utilized sites in June. B Includes additional sites utilized in July. C includes additional sites utilized in August. See Table I for factor loadings.

by broods occurred within <8 ha of the marsh and 88% (n = 73) of sightings of broods were at 3 sites which encompassed an area of 2 ha.

Analysis of the sample sites within this 5 m wide 8 ha zone indicated that the highest frequency of occurrence of ducklings was in the northeast corner of the marsh (sites 1-8, 20-22, 33 and 41) (Fig 2). When pooled, brood distributions observed during 93 transects produced a value of Morisita's index of dispersion of 0.540 (a value of > 0.5 indicates randomness with 95% confidence). When analyzed on a monthly basis, similar results were found (June 0.5306, n = 36; July 0.585, n = 32; August 0.5850, n = 24). Median tests showed that ducklings occurred most frequently at sites 1, 2 and 7 during June, at site 20 during July, at sites 1, 20 and 41 during August.

The results of PCA are summarized in Table I and Fig 3. It is clear that axis 1, which accounts for 16.2% of the total variation among sites, may be related to the presence of filamentous algae that made up the floating mat community. High positive loadings are found for algal genera *Ectocarpus, Enteromorpha, Capsosiphon, Rhizoclonium, Klebsormidium, Pilyella,* and *Blidingia,* which often occur together in floating mats (accompanied by blue-green algae). The subjective shelter value also shows a strong positive loading on PCA axis 1. Ordination of the sample plots (Fig 3) shows that two relatively distinctive groupings of utilized sites may be discerned along axis 1. Fig 2 shows that group A (sites 1-7) was utilized primarily during the first 3 weeks of brood rearing (June), whereas group B (sites 7, 20 and 21) showed large increases in duckling occupancy in July. This was particularly evident in site 20, which, through its position on axis 1, has many of the algal species that contribute to floating masses. Visible algal mats were first noted in mid to late June and their appearance was shown to correspond with the shift from group A (low algal presence) to group B sites (high algal presence).

Discussion

Courcelles and Bédard (1979) demonstrated that broods of black ducks and other species of ducks were four times more abundant in preferred (vs next best) locations in freshwater marsh habitat. They linked distribution of ducklings in five habitats to distribution of aquatic vegetation, and they further determined the ecological variables (water depth, available phosphorus, organic matter, pH, etc.) that correlated with plant distribution. Our observations show that all foraging activity by black duck ducklings in this study occurred within a narrow zone at the edge of the estuarine marsh. There was a high (88% of observations at 3 sites) frequency of use of specific sites within this zone. Indeed, only a very small area of the marsh was used. MacLean (1988) found that all (168 h observation, 187 sightings of broods) foraging of black duck broods in 1987 occurred within the 5 m zone, 71/79% at 7 sites of ≃ 4 ha; 52% occurred at 3 sites of ≃ 2 ha. The analyses in this study suggested that foraging activity was linked to the presence of floating mats of a composite of algal species. Although the amount of time spent at sites differed in this study from MacLean's (1988), the same sites were used by ducklings, and algal mats were the common denominator, though no specific algal species occurred throughout the sites.

Bengtson (1972) found food to be a critical factor influencing the pattern of distribution of duck broods and Hickey and Titman (1983) found that feeding was the major activity of black duck broods in Prince Edward Island. It is well known that the diet of ducklings consists of a high component of invertebrate matter (Collias and Collias, 1963; Bengtson, 1972; Sugden, 1973; Reinecke, 1979). Although the vegetative parts of aquatic plants are believed to be poor food for young waterfowl, it is indirectly important because of the high densities of macroinvertebrates typically associated with it. Berg (1949) and McGaha (1952) concluded that a variety of aquatic insects are restricted to one or a few closely related species of plants with floating or emergent parts. Krull (1970) confirmed a macroinvertebrate - submerged aquatic macrophyte association.

Our observations showed that a shift occurred in use of sites by ducklings after they were approximately three weeks old, from a sheltered northeastern pond to specific sites where floating mats of algae were found. MacLean (1988) reported a similar shift in habitat use by ducklings of this age to areas within the zone characterized by floating mats of algae, although she did not monitor the development of these mats. While algal mats are undoubtedly important to foraging ducklings, the presence of an extensive stand of dense emergent vegetation provides shelter from weather, and perhaps more significantly from the frequent avian predator attacks that occur during the first few weeks that broods are out on this marsh.

During 1986, the survival rate of ducklings in 16 black duck broods on this marsh was 95% after ducklings had reached 2 weeks of age, as opposed to <60% for ducklings during the first 2 weeks of life. Predation was the main source of duckling mortality. It is probable that this extensive zone, where both cover and food can be obtained, is the most important feature attracting females to this habitat for brood rearing.

Natural habitats that support algal mats appear to be suitable for brood rearing. Physical/biotic factors that increase the development of algal mats in association with emergent vegetation, should enhance the quality of habitat for brood rearing. While the foraging benefits appear obvious, cover from predators may be equally important (Seymour personal observation). Any management plan designed to concentrate broods in high nutrient areas must also provide suitable cover, and an extensive length of indented emergent edge should provide this.

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SYNTHESIS AND CHARACTERIZATION OF A DERIVATIVE OF 2-L-N-(L-GLUTAMYL-L-GLUTAMINYL) AMINO-3-PHENYLPROPAN-1-OL A PROPOSED C-TERMINAL PEPTIDE SEQUENCE OF METABOLITES OF TRICHODERMA SPP.

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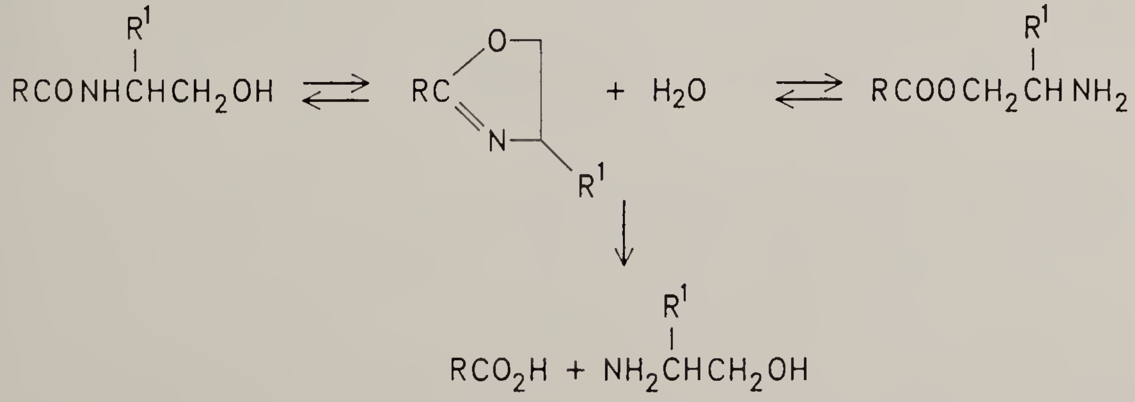
The γ-benzyl ester of the 2,4-dinitrophenyl ether of 2-L-N-(L-glutamyl-L-glutaminyl) amino-3-phenylpropan-1-ol, the C-terminal sequence of a number of antibiotic fungal peptides, has been synthesized. Evidence is presented that rearrangements did not occur at either of the two peptide bond forming steps. However, 2-L-pyroglutamylamino-3-phenyl-1-(2′, 4′-dinitrophenoxy)propane was always a byproduct in the reaction of the glutamylphenylalaninol ether with γ-benzyl N-t-butyloxycarbonylglutamic acid. The pyranyl ether protecting group of L-2-(N-L-t-butyloxycarbonyl-glutamyl)amino-3-phenyl-1-(tetrahydropyranyl-2′-)oxypropane could be selectively hydrolysed with lithium tetrafluoroborate.

On a synthésizé l'ester γ-benzyl de l'éther 2,4-binitrophenyl de 2-L-N-(L-glutamyl-L-glutaminyl) amino-3-phenyl-l-ol, la sequence terminale-C d'un nombre de peptides de mycètes antibiotiques. Les faits presentés ici démontrent que les réarrangements ont lieu à nulle étape où deux peptides forment des liaisons. Néanmoins, le propane 2-L-pyroglutamylamino-3-phenyl-1-(2′,4′-binitrophenoxyl) a constitué un dérivé constant de la réaction de l'éther glutamylphenylalaninol avec l'acide γ-benzyl N-t-butyloxycarbonylglutamique. On a réussi à hydrolyser, au moyen du tetrafluoroborate de lithium, le groupe protegeant l'éther pyranyl du propane-L-2-(N-L-t-butyloxycarbonylglutamyl) amino-3-phenyl-1-oxy(tetrahydropyranyl-2′).

The peptide (**1**, $R=R'=R''=H$) is thought to be the C-terminal sequence of a large number of antibiotics produced by several species of fungi (Shaw & Taylor, 1986). A considerable effort has been directed to its synthesis which has been reported by four independent groups of workers (Nagaraj & Balaran, 1981; Gisin et al., 1981; Balasubramanian et al., 1981; Schmitt & Jung, 1985). However the physical properties of the four products reported by these workers differ by amounts greater than is normally attributable to experimental error (Shaw & Taylor, 1986). In two of these syntheses (Gisin et al., 1981; Balasubramanian et al., 1981) the compound (**2**, $R=H$, $R'=t\text{-}C_4H_9OCO$) was used as an intermediate and here again the melting points (m. p. 134°, 146°) and optical rotations ($[\alpha]_D$ -39°, -31°) of this intermediate differed.

Scheme 1



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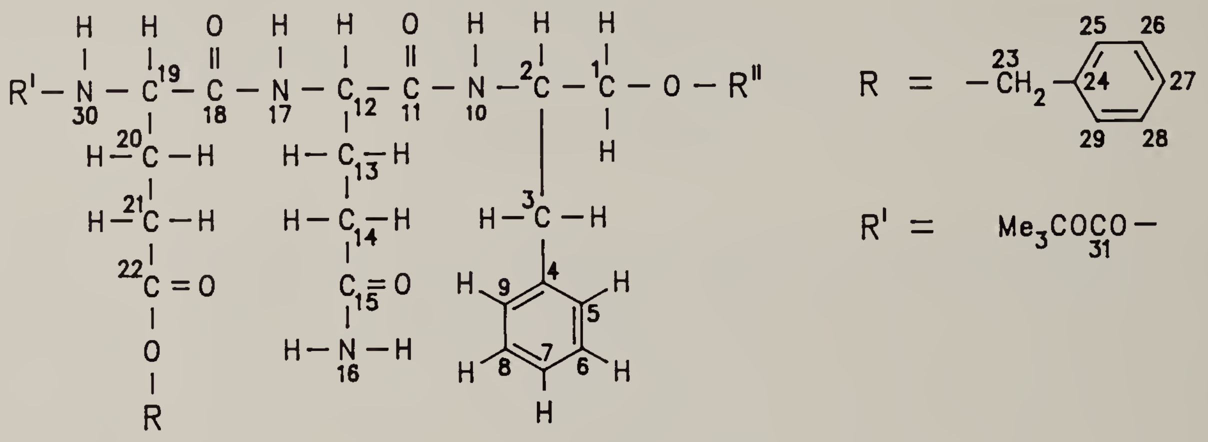
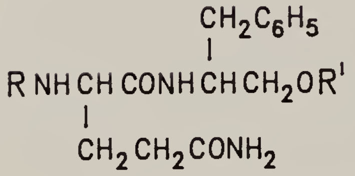
Acylaminoalcohols are known (Frump, 1971) to cyclise to oxazolines as shown in scheme 1 under a variety of reaction conditions and a mechanism involving an intermediate oxazoline has been proposed for the ready release of the phenylalaninol residue from a mixture of alamethicins (Martin and Williams, 1976). It was therefore appropriate to attempt to synthesise the compound **2** under conditions where the hydroxyl group of the phenylalaninol residue was protected, and thus unable to participate in oxazoline formation.

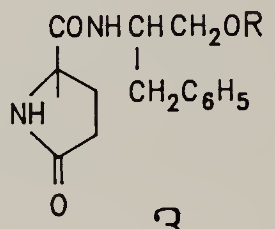


1



$$\begin{array}{c} CH_2C_6H_5 \\ | \\ RNHCHCONHCHCH_2OR' \\ | \\ CH_2CH_2CONH_2 \end{array}$$

2



3

L-phenylalaninol readily condensed with dihydropyran to give a diasterioisomeric mixture of basic tetrahydropyranyl ethers, characterized as their 4-chlorobenzoyl derivatives. This mixture of bases on treatment with L-t-butyloxycarbonylglutamine gave a mixture of pyranyl ethers (**2**, R'=t-C_4H_9OCO, R=C_5H_9O). Under a wide range of acidic conditions these ethers gave the pyroglutamic acid derivative (**3**, R=H), but on treatment with lithium tetrafluoroborate (Lipshutz & Harvey, 1982; Taylor & Reiter, 1989), a 50% yield of the alcohol (**2**, R'=t-C_4H_9OCO, R=H) was obtained. This alcohol was characterized by conversion to its acetate, its pyranyl ethers, and as its dinitrophenyl ether. This dinitrophenyl ether was eluted as a Gausian shaped peak from a reversed phase chromatography column with a retention volume of 33.3 mL.

The structure of the dinitrophenyl ether was established from the combined ^{13}C and ^{1}H n.m.r. data as follows. A ^{1}H 2-dimensional correlation spectrum (COSY) gave the ^{1}H spin-spin coupling connectivities within each residue, viz H'_1, H_2, H_3, H_{10}, and H_{12}, H'_{13}, H'_{14}, H_{17} (the numbering system for the various nuclei is given on formula **1**, for compound **2** it is analogous) which subsequently permitted δ_H and J_{HH} for these protons to be determined by simulation. Resonances of the t-C_4H_9OCO group were assigned by their integrated intensities, chemical shifts and lack of long-range coupling. The resonances of the aromatic carbons C_4 to C_9 were assigned with the aid of a ^{1}H-coupled ^{13}C spectrum in which C_5, C_7, and C_9 each showed 3-bond coupling to 2 protons, while C_6 and C_8 were 3-bond coupled to 1 proton. Relative integrated intensities of the ^{13}C resonances distinguished C_7 from C_5 and C_9, these carbons being likely to have comparable relaxation times. The quaternary carbons of the dinitrophenyl group were assigned from long-range couplings and calculations of substituent effects (Breitmezer and Voelter, 1987): predicted δ_C for C_1 to C_6 of dinitrophenyl 161.8, 136.0, 120.9, 142.6, 131.4, 116.7; observed 157.2, 138.9, 122.2, 141.4, 129.7, 116.2. A ^{13}C-^{1}H heterocorrelation experiment indicated the ^{13}C assignments of all carbons bearing protons with distinguishable chemical shifts, the glutamine peptide CO being typically at lower field than its amide CO (Wuthrich, 1987). Finally, ^{13}C isotopic shifts, measured after exchange of N-H protons with ^{2}H and careful drying of the product before dissolving it in tetrahydrofuran (THF $^{2}H_8$) gave the following results (p.p.m., negative sign indicates upfield shift): C_{15} (-0.062), C_{11} (-0.083), C_{18} (-0.017), C_2 (-0.086), C_3 (-0.027), C_{14} (-0.049), and C_{13} (-0.052). All other shifts were either downfield or less than 0.003 p.p.m., 10 peaks changing by less than 0.001 p.p.m. Such a pattern of shifts supports the structure (**2**, R=t-C_4H_9OCO, R′=$C_6H_3(NO_2)_2$); and does not support products obtained by rearrangement via an oxazoline intermediate.

The pyroglutamyl derivative (**3**, R=$C_6H_3(NO_2)_2$) was always a component of the products obtained from solvolytic reactions of this dinitrophenyl ether (**2**, R=$C_6H_3(NO_2)_2$, R′=C_4H_9OCO). The conditions given in the Experimental section, the results of a large number of orientation experiments, are those where the minimum formation of pyroglutamate was observed. Condensation of the crude solvolytic reaction mixture with γ-benzyl t-butyloxycarbonyl-L-glutamate (Sanderin and Boissonas, 1963) under the conditions described by Neubert and Jakubke (1978) gave the tripeptide (**1**, R=C_7H_7, R′=t-C_4H_9OCO, R″=$C_6H_3(NO_2)_2$) in about 60% yield where racemisation of the glutamine residue was minimal.

Though this product could be obtained as a crystalline solid, it proved unsuitable for X-ray diffraction analysis. Solutions (1%) in warm methyl, ethyl and butyl alcohols, acetone and ethyl acetate formed rigid gels on cooling to room temperature. The structure was confirmed by comparison of the n.m.r. spectra with those of (**2**, R=t-C_4H_9OCO, R′=$C_6H_3(NO_2)_2$). All resonances of corresponding nuclei were readily identifiable and the remainder were assigned by ^{1}H COSY, ^{1}H nuclear Overhauser effects (nOe), ^{1}H-^{13}C heterocorrelations and ^{1}H-coupled ^{13}C spectra. Coupling constants and chemical shifts for H'_1, H_2, H_3 and H_{10}, and for the overlapping resonances of the glutamic acid and glutamine residues, were obtained by spectral simulation, thus overlapping resonances of protons and directly bonded carbons were assigned unequivocally. The resonances of the aromatic carbons C_{24} to C_{29} (see Fig 1) were assigned from multiplicities in the ^{1}H-coupled ^{13}C spectrum as described for the phenyl rings in compound **2**. The lowest field signal, δ_C 175.22 and those at δ_C 172.25 and 172.44 were broad singlets in the coupled spectrum, and have counterparts in the spectrum of (**2**, R=t-C_4H_9OCO, R′=$C_6H_3(NO_2)_2$). They were therefore assigned respectively to C_{15}, and C_{11} or C_{18}. The remaining signal at 173.08 p.p.m., which showed long-range coupling to hydrogen substituents of at least 6 other carbon nuclei, is therefore C_{22}. Independent confirmation of the peptide sequence was obtained by ^{1}H nOe-difference measurements. Irradiation of the N-H resonances at $\delta_{H_{30}}$ 6.52, $\delta_{H_{17}}$ 8.023 and $\delta_{H_{10}}$ 7.89 produced nuclear Overhauser effects as follows: δ_H 6.52 enhanced H_{19}, $\delta_{H_{17}}$ 8.02 enhanced H_{12} and H_{19}, and δ_H 7.89 enhanced H_2 and H_{12}, in accord with structure **1**.

Thus the structure of the tripeptide **1** is firmly established and this compound will therefore serve as a point of reference in future synthetic studies in this field.

Experimental

Melting points are not corrected. Thin layer chromatography (tlc) was done on silica gel plates (Merck) and high pressure liquid chromatography (hplc) on reversed phase columns used ammonium acetate pH 4.2 buffer-methyl alcohol mixtures as the developing solvent (proportions given for each compound). Optical rotations were measured using a Perkin-Elmer 141 polarimeter, infrared spectra on a Perkin Elmer 237 instrument and ultraviolet spectra on a Unicam SP8000 spectrometer. Nuclear magnetic resonance (n.m.r.) spectra were obtained at 20° in tetrahydrofuran (THF-$^2H_8$) or methyl alcohol [$^2H_4$] as indicated in the text. Spectra were recorded on a Bruker MSL-300 or a Nicolet NB-360 (Atlantic Magnetic Resonance Center) instruments at 300 or 360 MHz for proton spectra and 75.5 or 90.8 MHz for ^{13}C spectra. Data tables up to 64K were used where necessary. All chemical shifts are given in p.p.m. downfield from the signal of tetramethylsilane. Coupling connectivity was determined by ^1H COSY and $^{13}C/^1H$ heterocorrelation spectra. ^1H Spectral simulations using the Bruker PANIC programme gave ^1H chemical shifts and coupling constants and confirmed coupling patterns where necessary. Assignments are based on this coupling information, known chemical shifts of ^1H and ^{13}C nuclei in amino acid residues in peptides (Breitmezer & Voelter, 1987; Wuthrich, 1987), substituent effects for aromatic rings (Wuthrich, 1987), longrange coupling to quaternary carbons and isotope effects on ^{13}C chemical shifts produced by ^2H exchange. Chemical shifts and coupling constants are presented with reference to an arbitrarily numbered formula (**1**). Unless stated otherwise chemical shifts refer to single protons, and the numbering of the latter refers to the nitrogen or carbon atom whose substituent they are. All other n.m.r. data are given in the format used by Shaw & Taylor (1986).

2-(L-2′-Amino-3′-phenylpropan-1′-oxy)tetrahydropyranyl ethers - L-2-Amino-3-phenylpropan-1-ol (10 g, 66 mmol) was dissolved in hydrochloric acid (*N*, 85 mL) and the solution was evaporated. The crude hydrochloride (12.4 g) in chloroform (300 mL) was treated with dihydropyran (18.8 mL, 206 mmol) and toluene-4-sulphonic acid (90 mg, .5 mmol). The solution was kept for 9 h at room temperature when it was washed with sodium carbonate solution (5%, 2 x 120 mL) and saturated brine (100 mL). The dry (Na_2SO_4) chloroform solution was evaporated to give an oil (17.7 g). This oil (1.8 g) was applied to a silica gel column (100-200 mesh, packed with diethyl ether, 4.5 x 27 cm) which was developed with diethyl ether (500 mL), diethyl ether-methyl alcohol (49:1, 500 mL), diethyl ether-methyl alcohol (24:1, 500 mL) and diethyl ether-methyl alcohol (19:1, 500 mL). The pyranyl ethers (0.7 g) were collected in the fractions eluted with the last 2 solvents and were purified for analysis by distillation; b. p. 120-130°/0.1 mm Hg, (Found, C, 71.1; H, 8.8; N, 5.85; O, 13.5. $C_{14}H_{21}NO_2$ requires C, 71.4; H, 9.0; N, 5.95; O, 13.6%), pK_a 8.1 (E. Wt. 243), $[\alpha]^{25}_D$ +7° (c, 3, MeOH), $\lambda_{max.}$ (KBr) 1603, 1495, 1200, 1030 cm^{-1}, δc (C^2HCl_3) 139.0 (q. arom. C), 129.2 (2C, arom.), 128.6, 128.3, 126.2, 99.4 (ketal CH), 72.5 (propanol CH_2), 62.3 (pyranyl-2 CH_2), 52.4 (propanol CH), 40.9 (benzyl CH_2), 30,6, 25.5, 19.6. This amine (121 mg) in THF (5 mL) was treated with 4-chlorobenzoyl azide (Shaw & Taylor 1986, 100 mg) and the resulting solution kept at 20° for 4 days. The solution was then evaporated, the residue digested with several portions of petroleum ether (b. p. 30-60°). The petroleum ether was decanted after each digestion. *(2-(L-2′-(N-4″-chlorobenzoylamino)-3′*-phenyl-propan-1′-oxy) tetrahydropyranyl ethers separated from isopropyl ether as needles, m. p. 118-129°, R_v (3:17) 10.4 and 11.2 mL, (Found, C, 67.65; H, 6.5; Cl, 9.3; N, 3.7; O, 12.9. $C_{21}H_{24}ClNO_3$ requires C, 67.5; H, 6.5; Cl, 9.5; N, 3.75; O, 12.8%), $[\alpha]^{22}_D$ -41° (c, 1.3, MeOH), υ_{max} (KBr) 1640, 1600, 1500 cm^{-1}, δ_H (C^2HCl_3) 7.72 (2H, $^3J_{HH}$ 8.5 Hz, $^4J_{HH}$ 3 Hz), 7.32 (2H, $^3J_{HH}$ 8.5 Hz), 7.25 (5H), ca. 4.6 (2H, m), ca. 3.7 (4H, m), 3.6 (2H, $^2J_{HH}$ 7 Hz), ca. 1.6 (6H, m).

L-2-(N-2′-(N′-t-butyloxycarbonyl)-L-glutaminyl-amino-3′-phenylpropan-1′-oxy) tetrahydropyranyl ethers - (a) The tetrahydropyranyl ethers described in the previous paragraph (8.62 g 36.6 mmol), N-t-butyloxycarbonyl-L-glutamine (Hofmann et al., 1965, 9.02 g, 36.6 mmol), 1-ethoxy-carbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, 9.93 g, 36.5 mmol), and toluene (300 mL) were stirred together at 35° for 4 days. The reaction mixture was cooled to 4° and kept at this temperature for 1 h when the precipitate (13.5 g, m. p. 130-150°, 80%) was collected. The *L-glutaminyltetrahydropyranyl ethers* (**2**, R=C_5H_9O, R′=C_4H_9OCO) separated from ethyl acetate as rods, m. p. 132-153°, R_v 6.9 and 7.7 mL (3:17), (Found, C, 62.4; H, 8.0; N, 9.05; O, 21.0. $C_{24}H_{37}N_3O_6$ requires C, 62.2; H, 8.0; N, 9.1; O, 20.7%), $[\alpha]^{21}_D$ - 25.0° (c, 2.3, $CHCl_3$), $\upsilon_{max.}$ (KBr) 1660, 1550, 1520, 1250 cm^{-1}, δ_C (C^2HCl_3) 175.2 (175.15), 171.1 (170.0), 155.9 (urethane CO), 138.1, 129.4 (2C, $^1J_{CH}$ 160 Hz), 128.4 (2C, $^1J_{CH}$ 160 Hz), 126.4 ($^1J_{CH}$ 159 Hz), 100.2 (99.3) ($^1J_{CH}$ 160 Hz), 79.99 (t-Bu C), 68.3 (68.0) J_{CH_2} 142 Hz, benzyl), 63.3 (62.7) ($^1J_{CH_2}$ 139 Hz), 52.4 (b, CH_2), 50.8 (50.35) (J_{CH_2} 136 Hz), 37.8 (37.5), 31.8, 30.8 (30.6), 29.3 (29.1), 28.35 (t-butyl), 25.3, 20.1 (19.7) (J_{CH_2} 131 Hz).

(b) L-2-(N-(N′-t-butyloxycarbonyl-L-glutaminyl)amino-3-phenylpropan-1-ol (see below, 100 mg, 0.26 mmol), chloroform (5 mL) and dihydropyran (0.08 mL, 0.94 mmol) were mixed together and the mixture treated with a solution (3 mL) of toluene-4-sulphonic acid (1 mg) in chloroform. The mixture was stirred at 20° for 4 h, was then diluted with chloroform (50 mL) and the solution washed with sodium bicarbonate solution (5%, x2) and brine. The dry (Na_2SO_4) chloroform solution was evaporated and the residue (120 mg) recrystallized from ethyl acetate gave the tetrahydropyrenyl ethers (110 mg, m.p. 140-150°, 90%) identical with the material prepared as described in the preceeding paragraph.

L-2-(N-L-pyroglutamyl)amino-3-phenylpropan-1-ol - (a) The tetrahydropyranyl ethers, prepared in the preceeding paragraph (**2**, R=C_5H_9O, R′=t-C_4H_9OCO, 1 g, 2.15 mmol) in THF (50 mL) were treated at 0° with a slow stream of hydrogen chloride for 15 min. Excess hydrogen chloride was removed with a stream of nitrogen, the precipitate collected, washed with ice-cold THF and dried. *2-L-N-Pyroglutamylamino-3-phenylpropan-1-ol* separated from ethyl alcohol as colorless needles m.p. 173-175°, R_v 3.85 (3:17), (Found, C, 64.05; H, 5.9; N, 10.7). $C_{14}H_{18}N_2O_3$ requires C, 64.1; H, 6.9; N, 10.7%), $[\alpha]^{23}_D$ - 39° (c, 2, MeOH), $\upsilon_{max.}$ (KBr) 1695, 1655, 1560, 1045 cm^{-1}, δ_H ($C^2H_3O_2H$) 7.23 (5H), 4.25-4.0 (2H, m), 3.65 (2H, CH_2OH), 2.95-2.70 (2H, benzyl CH_2), 2.4-2.0 (4H, m).

(b) L-pyroglutamic acid (1.29 g, 10 mmol), L-2-amino-3-phenylpropan-1-ol (1.51 g, 10 mmol), EEDQ (2.72 g, 11 mmol), and toluene (100 mL) were stirred together at 45° for 4 days. The reaction mixture was cooled to 4°, kept at this temperature for 18 h and the colorless crystalline precipitate collected. The pyroglutamylphenylalaninol was recrystallized from ethyl alcohol as needles m. p. 174°, $[\alpha]^{22}_D$ - 38° (c, 1.5, MeOH), 2.48 g, 94%.

L-2-(-N-(N′-t-Butyloxycarbonyl-L-glutaminyl)amino-3-phenylpropan-1-ol - The tetrahydropyranyl ethers (**2**, R=C_5H_9O, R′=t-C_4H_9OCO, 2.32 g, 5 mmol) were dissolved in a solution (20 mL) of water (0.4 mL) in acetonitrile by heating the mixture to 45°. The solution was cooled to 15° and was then treated with a solution (5 mL) of lithium tetrafluoroborate (98%, 0.467 g, 5 mmol) in aqueous acetonitrile (2%, v/v). The resulting solution was kept at room temperature for 72 h, was then evaporated at <10° to ca. 5 mL and the concentrate diluted with ice-cold saturated brine (50 mL). The mixture was extracted with ethyl acetate (5 x 50 mL) and the combined extracts washed with sodium bicarbonate soultion (5%, 2 x 20 mL). The dry (Na_2SO_4) extract was filtered and the filtrate evaporated. The residue (2 g) crystallized on tituration with ethyl acetate. The *phenylalaninol* (**2,** R=H, R′=t-C_4H_9OCO) separated from ethyl acetate as long thin rods m. p. (138°) 149-150°, R_v 5.8 mL (3:17), (Found C, 60.2; H, 7.7; N, 11.1; O, 21.1. $C_{19}H_{29}N_3O_5$ requires C, 60.1; H, 7.7; N, 11.1; O, 21.1%), $[\alpha]^{24}_D$ - 41° (c, 1.8, MeOH), $\upsilon_{max.}$ (KBr) 1680, 1660, 1525 cm^{-1}, δ_H (THF, $^2H_8$) 1.40 (9H), 1.85 ($^2J_{H_{13}H_{13}}$ -14.0 Hz, $^3J_{H_{13}H_{14}}$ 7.2 Hz, $^3J_{H_{13}H_{14}}$ 7.6 Hz, $^3J_{H_{13}H_{12}}$ 7.9 Hz), 1.95 ($^3J_{H_{13}H_{12}}$ 5.9 Hz, $^3J_{H_{13}H_{14}}$ 7.2 Hz, $^3J_{H_{13}H_{14}}$ 6.8 Hz), 2.15 ($^2J_{H_{14}H_{14}}$ -15.2 Hz), 2.19, 2.72 (2H, e), 2.77 ($^2J_{H_3H_3}$ -13.5 Hz, $^3J_{H_3H_2}$ 6.7

Hz), 2.85 ($^3J_{H_3H_2}$ 7.5 Hz), 3.38 ($^2J_{H_1H_1}$ -10.8 Hz, $^3J_{H_1H_2}$ 5.3 Hz), 3.45 ($^3J_{H_1H_2}$ 4.4 Hz), 4.02 ($^3J_{H_2H_{10}}$ 7.9 Hz), 4.03 ($^3J_{H_{12}H_{17}}$ 8.0 Hz), 6.35 (H_{17}, e), 7.11 (m H_7), 7.20-7.22 (4H), 7.45 (H_{10}, e), δ_C 28.66 (3C), 29.68 (C_{13}), 32.20 (C_{14}), 37.74 (C_3), 53.73 (C_2), 54.80 (C_{12}), 63.06 (C_1), 79.01 (Me_3C), 126.68 (C_7), 128.89 (C_6 & C_8), 130.21 (C_5 & C_9), 140.05 (C_4), 156.4 (BuO C O), 172.0 (C_{11}), 175.31 (C_{15}). This alcohol (0.2 g, 0.53 mmol) was suspended in acetic acid (0.35 mL) and the stirred suspension treated with acetic anhydride (1.13 mL, 12 mmol). The mixture was stirred for 4 days at room temperature, the resulting solution evaporated and the residue, recrystallized from ethyl *acetate* gave the acetate (**2**, R=Ac, R′=t-C_4H_9OCO) as needles m. p. 152-153°, (Found C, 59.9; H, 7.4; N, 10.0; O, 22.7. $C_{21}H_{31}N_3O_6$ requires C, 59.8; H, 7.4; N 10.0; O, 22.8%), $\upsilon_{max.}$ (KBr) 1720, 1680, 1665, 1560, 1525, 1275 cm^{-1}.

L-2(-N-(N′-t-Butyloxycarbonyl-L-glutaminylamino)-3-phenyl-1-(2′,4′-dinitro-phenoxy)propane - The glutaminylphenylalaninol (**2**, R=H, R′=t-C_4H_9OCO, 5.69 g, 15 mmol) was suspended in a solution (100 mL) of triethylamine (2.1 mL, 15 mmol) in acetone in the dark and under an atmosphere of nitrogen. A solution (30 mL) of 2,4-dinitrofluorobenzene (2.8 g, 15 mmol) was added and the stirred mixture heated under reflux for 4 days. The mixture was evaporated, the residue dissolved in ethyl acetate (650 mL) and the solution washed with dilute hydrochloric acid (5%, 250 mL), then with sodium bicarbonate solution (5%) until the aqueous phase was colorless and finally with brine. The dry (Na_2SO_4), filtered ethyl acetate solution was evaporated and the residue (8.1 g) taken up in hot ethyl alcohol (125 mL). The filtered solution was allowed to cool very slowly (ca. 5 deg. C h^{-1}) and after 18 h the crystalline solid (=A, 3.56 g, m. p. 159-167°) was collected. The residue (3 g) from evaporation of the mother liquors from this crystallization was dissolved in ethyl acetate (50 mL) and applied to a silica gel (180 g, 100-200 mesh) column. The column was developed with ethyl acetate and the first 50mL of eluate were discarded. The following 1.1 L of eluate were collected and evaporated to give yellow needles (1.14 g). The *dinitrophenyl ether* separated from ethyl alcohol as very fine yellow needles m. p. 164-168°, R_v 12.4 mL (1:5), (Found C, 55.4; H, 6.1: N, 12.8 $C_{25}H_{31}N_5O_9$ requires C, 55.0; H, 5.7: N, 12.8%), $[\alpha]^{24}_D$ -41° (c, 0.9, MeOH), $\lambda_{max.}$ (MeOH) 251,289 nm (ε 10800 15000), $\upsilon_{max.}$ (KBr) 1680, 1660, 1610, 1525, δ_H (THF $^2H_8$) 1.37 (9H), 1.77 (H_{13}), 1.88 (H_{13}), 2.12 (H_{14}), 2.59 ($2H_{16}$, e) 2.98 (H_3), 3.19 (H_3), 4.02 (H_{12}), 4.20 (H_1), 4.31 (H_1), 4.42 H_2, 6.20, 6.38 (H_{17}, e), 6.65, 7.15 (H_7), 7.12-7.30 (4H), 7.47 (H_6 of 2,4-dinitrophenyl=DNP), 7.73 (H_{10}, e), 8.43 (H_5 of DNP), 8.77 (H_3 of DNP), δ_C (THF, $^2H_8$) 28.6 (3C), 29.52 (C_{13}), 32.23 (C_{14}), 37.39 (C_3), 50.93 (C_2), 54.93 (C_{12}), 71.29 (C_1), 79.09 ($Me_3C.OCO$), 116.17 (C_6 of DNP), 122.22 (C_3 of DNP), 127.21 (C_7). 129.22 (C_6 & C_8), 129.74 (C_5 of DNP), 130.12 (C_5 & C_9), 138.91 (C_2 of DNP), 140.08 (C_4), 141.40 (C_4 of DNP), 156.44 (C_{18}), 157.23 9C_1 of DNP), 172.60 (C_{11}), 174.83 (C_{15}). Further elution of the column with ethyl aceate-methyl alcohol (49:1, 200 mL) gave the starting alcohol (m. p. (138°) 150°, 0.83 g).

L-3-Phenyl-2-L-pyroglutamylamino-1-(2′, 4′-dinitrophenoxy)propane - 2-L-N-Pyroglutamylamino-3-phenylpropan-1-ol (83 mg), 0.32 mmol) was suspended in acetone (3 mL) and triethylamine (32 mg, 0.33 mmol) was added A solution of dinitrofluorobenzene (59 mg, 0.33 mmol) in acetone was added and the mixture heated in the dark in an atmosphere of nitrogen under reflux for 5 days. The reaction mixture was evaporated, the residue dissolved in ethyl acetate (20 mL) and the solution washed with sodium carbonate (5%) solution until the washings were colorless. The dry (Na_2SO_4) ethyl acetate solution was filtered, evaporated and the residue obtained (69 mg) was applied to a silica gel column (100-200 mesh, 25 g). The column was developed with ethyl acetate; the first yellow band eluted in 60 mL provided 2,4-dinitrofluorobenzene and the second 60 mL of eluate gave the *dinitrophenyl ether* which separated from methyl alcohol as yellow needles m. p. 200-201°, R_v 7.5 mL

(3:17), (Found C, 55.9; H, 4.7; N, 13.0; O, 25.9. $C_{20}H_{20}N_4O_7$ requires C, 56.1; H, 4.7; N, 13.1; O, 26.1%), $[\alpha]^{22}_D$ -39.5° (c, 0.54, dimethylsuphoxide).

L-2-(γ-Benzyl N-t-butyloxycarbonyl-L-glutamyl-L-glutaminylamino)-1-(2′,4′-dinitrophenoxy)-3-phenylpropane - The t-Butyloxycarbonyldipeptide (**2** R=$C_6H_3(NO_2)_2$, R′=t-C_4H_9OCO, 2.73 g, 5.9 mmol) was dissolved in a solution (38 mL) of concentrated hydrochloric acid in acetic acid (1:19). After 1 h at 20° the solution was evaporated at <20° (1 mm) and the residue was kept at 4° for 18 h at 1 mm pressure over sodium hydroxide pellets. The residue and γ-benzyl N-t-butyloxycarbonyl-L-glutamate (Sanderin & Boissonnas, 1963, 1.68 g, 5 mmol) were dissolved in THF (50 mL). The resulting solution was treated with triethylamine (0.7 mL, 5 mmol) and was stirred for 10 min at 0° when dicyclohexylcarbodiimide-pentafluorophenol complex (Kovacs et al., 1967, 1:3, 4.25 g, 5.2 mmol) was added. The reaction mixture was stirred at 0° for 2 h and was then kept at 4° for 70 h, when it was filtered and the filtrate evaporated. The residue was taken up in ethyl acetate (1 L), the solution washed with dilute sodium bicarbonate (2%, 2 x 250 mL) and then with saturated brine (250 mL). The combined aqueous phases were washed with ethyl acetate, the ethyl acetate solutions dried (Na_2SO_4), filtered and evaporated. The crude residues from 2 runs were dissolved in methyl alcohol (50 mL) and the warm solution absorbed onto silica gel (100-200 mesh, 13 g). The methyl alcohol solution was evaporated and the residue packed on the top of a silica gel column (135 g, 3 x 30 cm). The column was developed with ethyl acetate (650 mL) and then with methyl alcohol-ethyl acetate (1:49). The product (**1**, R=benzyl, R′=C_4H_9OCO, R″=2,4-dinitrophenyl, 2.62 g) was eluted in the first 1 L of the latter solvent and was recrystallized from methyl alcohol to give 2.05 g of a yellow powder m. p. 152-155°. Recrystallization of this material (30 mg) from n-butyl alcohol (3 mL) gave very fine yellow needles m. p. 153°, (Found C, 57.8; H, 5.8; N, 10.9; O, 25.3. $C_{37}H_{44}N_6O_{12}$ requires C, 58.1; H, 5.8; N, 11.0; O, 25.1%), $[\alpha]^{24}_D$ -26.3° (c, 1.8, THF), $\lambda_{max.}$ (MeOH) 254, 291 nm (ε 7700, 10800), δ_H 1.41 (9H), 1.83 (${}^2J_{H_{13}H_{13}}$ -15.5 Hz, ${}^3J_{H_{13}H_{14}}$ 6.6 Hz, ${}^3J_{H_{13}H_{14}}$ 7.5 Hz, ${}^3J_{H_{13}H_{12}}$ 5.9 Hz), 1.94 (${}^3J_{H_{13}H_{14}}$ 6.6 Hz, ${}^3J_{H_{13}H_{14}}$ 6.6 Hz, ${}^3J_{H_{13}H_{12}}$ 7.9 Hz), 2.13 (${}^2J_{H_{14}H_{14}}$ -15.6 Hz), 2.20 (H_{14}), 1.85 (${}^2J_{H_{20}H_{20}}$ -13.8 Hz, ${}^3J_{H_{20}H_{21}}$ 7.7 Hz, ${}^3J_{H_{20}H_{21}}$ 7.2 Hz, ${}^3J_{H_{20}H_{19}}$ 8.0 Hz), 2.02 (${}^3J_{H_{20}H_{21}}$ 7.7 Hz, ${}^3J_{H_{20}H_{21}}$ 7.2 Hz, ${}^3J_{H_{20}H_{19}}$ 5.7 Hz), 2.41 ($2H_{21}$), 2.70 ($2H_{16}$, e), 2.95 (${}^2J_{H_3H_3}$ -13.7 Hz, ${}^3J_{H_3H_2}$ 7.3 Hz), 3.13 (${}^3J_{H_3H_2}$ 7.3 Hz), 4.00 (H_{19}), 4.19 (${}^2J_{H_1H_1}$ -9.8 Hz, ${}^3J_{H_1H_2}$ 4.2 Hz), 4.30 (${}^3J_{H_1H_2}$ 5.1 Hz), 4.43 (${}^3J_{H_2H_{10}}$ 7.9 Hz), 5.07 ($2H_{23}$), 6.36, 6.52 (e, ${}^3J_{H_{30}H_{19}}$ 7.2 Hz), 6.81, 7.14 H_7), 7.21-7.27 (m, $H_5H_6H_8H_9$), 7.26-7.35 (m, $H_{25}H_{26}H_{28}H_{29}$), 7.47 (${}^3J_{HH}$ 9.3 H_6 of DNP), 7.89 (e, ${}^3J_{H_{10}H_2}$ 7.85 Hz), 8.02 (e, ${}^3J_{H_{17}H_{12}}$ 7.4 Hz), 8.41 (H_5 of DNP), 8.74 (H_3 of DNP, ${}^4J_{HH}$ 2.8 Hz), δ_C 28.42 (C_{20}), 28.68 (3C), 28.84 (C_{13}), 31.08 (C_{21}), 32.23 (C_{14}), 37.31 (C_3), 51.00 (C_2), 53.84 (C_{12}), 55.08 (C_{19}), 66.54 (C_{23}), 71.20 (C_1), 79.39 (t-butyl), 116.16 (C_6 of DNP), 122.23 (C_3 of DNP), 127.15 (C_7), 128.62 (C_{27}), 128.86 (C_{25} & C_{29}), 129.11 (C_{26} & C_{28}), 129.18 (C_6 & C_8), 129.70 (C_5 of DNP), 130.12 (C_5 & C_9), 137.64 (C_{24}, by elimination), 138.98 (C_2 of DNP), 140.07 (C_4), 141.28 (C_4 of DNP), 156.80 (C_{31}), 157.25 (C_1 of DNP), 172.25 (C_{11} or C_{18}), 172.44 (C_{18} or C_{11}), 173.08 (C_{22}), 175.22 (C_{15}). The column was then eluted with methyl alcohol-ethyl acetate (1:19) and the next 800 mL discarded and the following 450 mL collected. This eluate was evaporated and the residue (0.166 g) recrystallized from methyl alcohol (5 mL) gave the pyroglutamic acid derivative (**3,** R=$C_6H_3(NO_2)_2$, 72 mg, m. p. 198-200°.

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THE BLUNTNOSE SIXGILL SHARK HEXANCHUS GRISEUS (BONNATERRE, 1788), NEW TO THE FISH FAUNA OF ATLANTIC CANADA

A young female specimen (NMC 90-0032) of *Hexanchus griseus* about 70.3 cm long (extreme tail tip damaged) was caught in a gill net set for pollock (*Pollachus virens* (Linnaeus)) at a depth of 155 m on 19/8/1990 at 44° 01′ N, 63′ 05′ W. A second male specimen (NSM 10001, Fig. 1) was similarly caught at 183 m on 22/10/1989 in Emerald Basin (44° 22′ N, 62° 42′ W). These two specimens are the first and second records of this species from Atlantic Canada and extend its range northward from North Carolina. The following is a description of the specimens with additional information from Bigelow & Schroeder (1948) and Compagno (1984).

Description Body stout anteriorly, tapering posteriorly and compressed posterior to pelvic fins. Head bluntly rounded and short. Caudal peduncle short and stout with dorsal fin base length entering 1.2-1.4 times in length from dorsal fin insertion to upper caudal fin origin. The ventral caudal peduncle length enters 1.8-1.9 times in the dorsal caudal peduncle length. No precaudal pits. Eye large and oval, horizontal diameter 4.9-5.2 times in head length. Mouth large and crescentic, length 0.6 times in width. Mouth width 0.4 times in preoral length. Upper lip covering rear part of lower jaw. Spiracle small, slightly above level of upper eye margin and slightly behind mouth corner level. Gill openings long, extending from a level with top of eye onto the ventral throat surface obliquely. The first gill opening is the longest and subsequent ones are progressively smaller. The gill arches are finely denticulate. Skin denticles have 3 horizontal crests terminating in points. On each side of the lower jaw there are 6 large teeth plus a median tooth with 4 central cusps in two pairs, each pair recurved laterally. The 6 large teeth are twice as wide as high and have 5-6 laterally-directed cusps. The cusps decrease in size laterally. Remaining lower jaw teeth are progressively smaller. In the upper jaw, the first 2 teeth have a single cusp and are slightly curved laterally. Subsequent teeth are larger, although they progressively decrease in size towards the mouth corner, have a stronger lateral curve, a broad base, a rounded basal cusp for 4 teeth which then becomes a pointed basal cusp for 3 teeth. Remaining upper jaw teeth are low and rounded with cusps weak to absent. The dorsal fin has a rounded tip, a weakly convex posterior margin and the rear tip is about half as long as the fin base and is triangular. The dorsal fin origin is just behind the cloaca level. The anal fin base is about equal to the dorsal fin base (anal base 0.9-1.3 times in dorsal base with the male having the larger dorsal fin base), the rear margin is almost straight and the rear tip is short. The anal fin origin lies under the middle of the dorsal fin base. The caudal fin is long, 2.8-2.9 times in total length. There is an obvious subterminal notch with a well-developed ventral lobe. The upper margin of the caudal fin is convex anteriorly and concave posteriorly. The posterior upper margin has larger denticles than the rest of the body. These denticles are smooth and oval and form a prominent edge to the fin. The lower margin is strongly concave posterior to the lower lobe. The pelvic fins have rounded tips and rounded margins. In the male the pelvic fin has an extended tip. Claspers lie in a dorsal groove of the pelvic fin and are black-tipped. The inner pelvic fin margins are attached anteriorly just posterior to the cloaca in the female. The pectoral fin is 1.7 times longer than broad, with a rounded tip, concave distal margin and an inner margin straight and 2.2-2.3 times in the anterior margin.

Preserved specimens have the back and upper sides grey, lower sides and abdomen white. A white lateral streak, up to 3.5 mm wide, runs from the head to near the end of the caudal fin on mid-flank. The streak dips sharply on the caudal fin base. The fins are grey.

Only two shark species have 6 gill openings. The bluntnose sixgill is distinguished from the bigeyed sixgill shark by having a short, broad blunt snout, six rather than five, rows of large, comb-like teeth on each side of the lower jaw, and the dorsal fin base length is about equal to or slightly longer than the distance from the dorsal fin insertion to the caudal fin origin.

The species is distributed world-wide in tropical and temperate seas. It is known in British Columbia but is rare on the Atlantic coast of North America, with a record from North Carolina in 1886, and records for Florida and the Gulf of Mexico.

Young are often closer inshore than adults which are found benthically below 91 m down to 1875 m. Adults of this species may rest on the sea bed during the day and rise to feed at night. Thus the small size of the specimens reported suggest that adults may be present in Nova Scotia waters. Generally regarded as sluggish, food nevertheless includes marlin, swordfish, sharks (including their own species), crustaceans, squids, seals and carrion. This species is ovoviviparous with 22-108 young.

Acknowledgements

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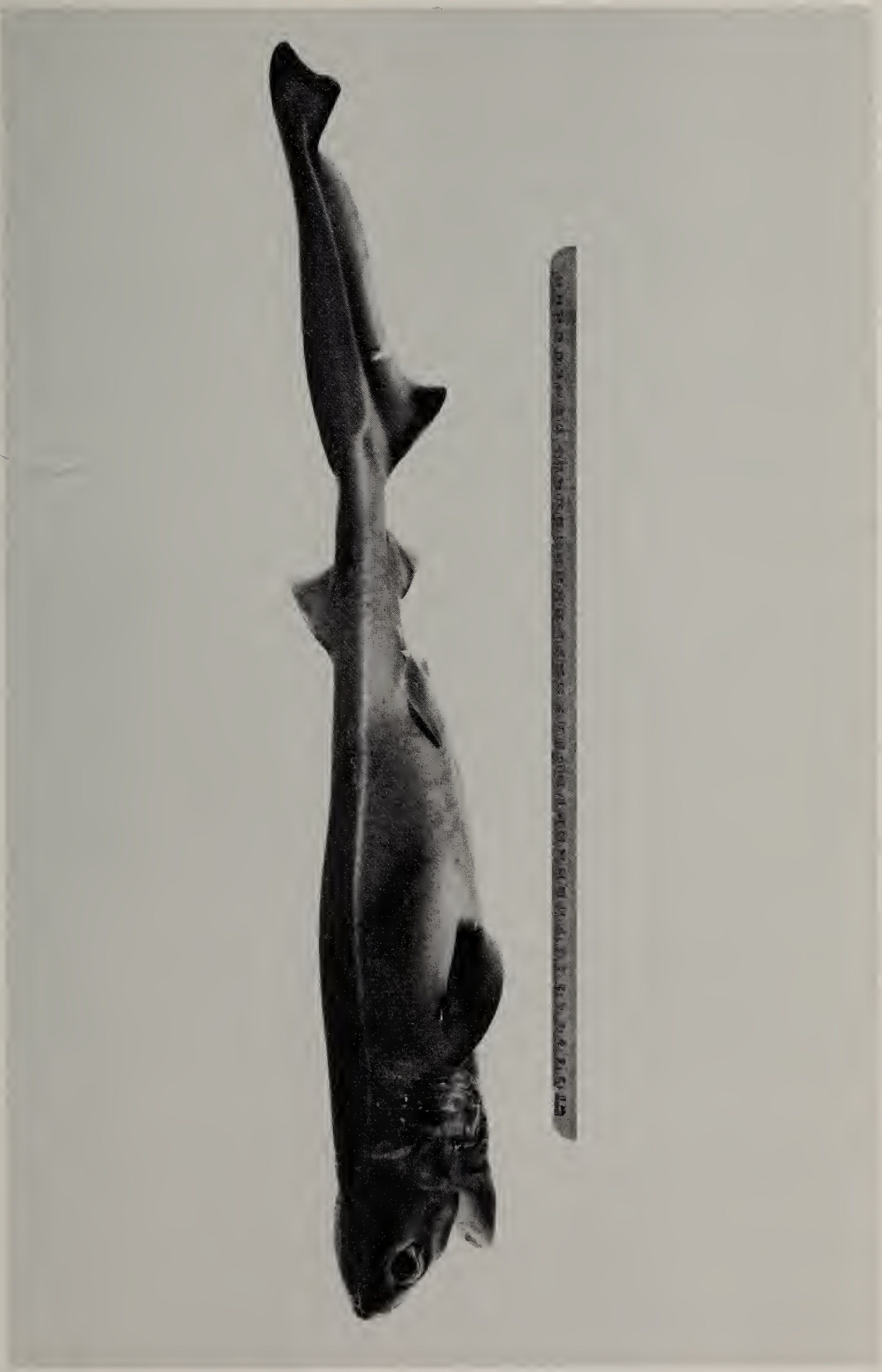

Fig 1 *Hexanchus griseus,* NSM 10001, from Emerald Basin, Nova Scotia.

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The *Proceedings of the Nova Scotian Institute of Science* publishes the proceedings of the Institute and original articles, including notes, pertaining to the natural sciences of the Atlantic Provinces, especially in biology and geology. Manuscripts may be either in English or French, and acceptance for publication is based on recommendations of referees.
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An abstract of not more than 200 words should precede the body of the text. This should be followed by an introduction, methods, results or observations, discussion, and references. In some cases a combination of these sections may be more effective.

References should be checked with the original article and referred to in the text by author and date, in parentheses. References should be listed alphabetically at the end of the manuscript. Reference to papers in periodicals must include author, date, title, journal, volume number, and inclusive pagination. Serials are abbreviated in the form given in **Bibliographic Guide for Editors and Authors** (the American Chemical Society, 1974). For serials not found therein, the abbreviated name in the **World List of Scientific Periodicals** (Butterworths, 1963) is used. The following are examples of style for references from journals, books, and symposium volumes:

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ASSEMBLY AND UTILIZATION OF A DATABASE OF FUNGAL CULTURES HELD IN CANADIAN COLLECTIONS[†].

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Descriptions of holdings of live cultures in ten Canadian collections of fungi have been collated and assembled as an indexed database. The data include the binomial names of the organisms, the date and place of their isolations, the substrate on which they were found, their mode of preservation, human pathology and ability to produce toxic metabolites. Each entry on the file has a unique accession number which indicates the collection where the organism is to be found. This file is indexed with respect to accession number and organism name and can therefore be searched efficiently for a particular organism, genus or species using simple commands. Access to the system, assembled on the Dalhousie University computer, has been freely available since 1987. At the moment it is not on line but is available to the public through the services of the Librarian of the Institute of Science. In four years we have had no evidence of abuse of the system by the public.

Les descriptions de dix collections canadiennes de cultures vivantes de mycètes ont été collationées et assemblées en base de données indexée. Les données comprennent les noms binominaux des organisms, la date et le lieu de leur isolement, la couche inférieure où ils ont été découverts, la manière de préservation, la pathologie humaine, et la capacité de produire des métabolites toxiques. Chaque inscription dans le fichier a un numero unique d'accession qui indique la collection où l'organisme est logé. Ce fichier est indexé quant au numero d'accession ainsi qu'au nom de l'organism; on peut alors le fouiller, avec des ordres minimes, pour un organisme particulier, un genre ou une espèce. L'accès à ce système, disposé sur l'ordinatuer à l'Université Dalhousie, est disponible sans frais depuis 1987. A l'heure actuelle, le système n'est pas branché, mais il est disponible par l'entremise des services de la bibliothécaire de l'Institute des Sciences. Pendant quatre années on a eu aucune évidence d'abus de ce système par le public.

Introduction

The Latin binomial names for living organisms, compiled since 1895 in the Index Kewensis are internationally accepted (among other things) as the code for retrieval of information about self-reproductive systems whether maintained as preserved specimens or as collections of viable species. Obviously such collections have many uses and are often assembled by students interested in groups of organisms having particular morphological or physiological characteristics. As Ainsworth (1961) has pointed out, such collections retain their value long after the reasons for their assembly have been submerged in printed scientific archives and after the death of their curators. There has, therefore, been a considerable effort to conserve these collections in institutions such as zoological and botanical gardens. These institutions have, with restrictions, permitted students access to the collections, but the value of such access depends on the holdings being readily known. Attempts to meet this need by publication of catalogues of specimens have been common, but have universally suffered from inaccuracy due to constant change in holdings. There have been many attempts to correct this deficiency by using mechanical search and retrieval systems. In theory, the ease of editing such data should make the systems up to date. In addition the availability of cheap personal computers and long distance telephone charges should make these databases available to a wide scientific audience.

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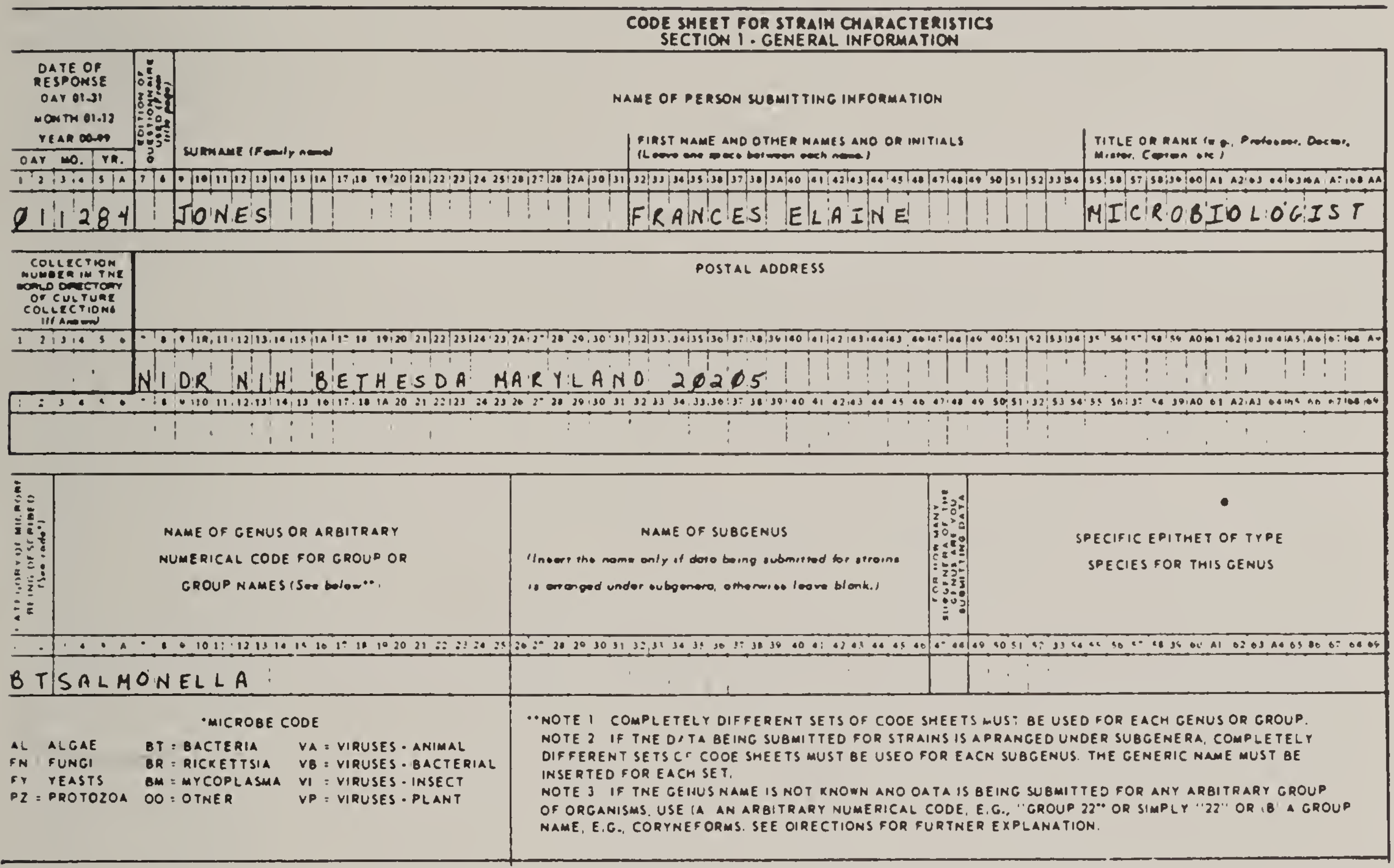
[†] NRCC No. 33018

One of the first attempts to implement this kind of database was that of Martin and his colleagues (Martin, 1964; Quadling and Martin, 1968; Simpson, et al., 1971). The database they assembled consisted of details of bacteria and some fungi held in about 60 collections of these organisms in Canada. They had the idea to organize the data in so-called fields i.e. blocks of specified numbers of bytes within a designated record assigned to each organism. Six fields were defined as follows: 1. Binomial name, authority, and its accession number in that collection. 2. the source of the organism, i.e. who deposited the organism in the collection. 3. where and from what the organism was originally isolated. 4. the methods of preservation in the collection. 5. 'special' features of the organism e.g. its pathogenicity. 6. references to the literature (if any). This system was used by bacteriologists in Canada and elsewhere but its main use was in compiling catalogues of organisms held in various collections and lists of names and addresses of such collections. As such it probably represented the first example of automated printing of microbiological data. Full details of search and editing programmes, files of synonyms of binomial names and other utilities have been published (Simpson et al., 1970). Its success also stimulated several industrial organizations holding collections of commercially valuable micro-organisms to automate retrieval of data about their collections in this manner.

It has collapsed because of lack of support, inaccessibility to working microbiologists, and the advent of more comprehensive databases. Two of these will be mentioned. The first operates, at least nominally, under the aegis of the American Type Culture Collection (ATCC) and is based on the work of Colwell and her colleagues (Rogosa et al., 1971, 1986; Philpot et al., 1982). This database was designed to be expandable i.e. as more information about particular species became available the field could be expanded without major modification in the search and retrieval programming. It was also designed to be much more comprehensive than the Martin database. This led to a system of converting information into a numerical six digit code, presumably convertible into machine readable code by scanning techniques. About 43 sections have been defined: 14 are broadly morphological and 12 strictly biochemical, though there is some duplication between sections. The first two sections of the code and section 16 contained information used in the Martin database but in more detail. For example the first section (Fig 1) contained the name and address of the person supplying the information, the genus, sub-genus and species epithet, a 'microbe code' of 12 two digit abbreviations for algae, fungi (& yeasts!), protozoa, bacteria (and rickettsia), mycoplasmas, virus (animal, insect!, bacterial & plant) and 'others'. In addition 20 questions (Fig 2) regarding strain characteristics are posed, the answers to many being apparent to a microbiologist from the preceding data. In addition to the 3 sections mentioned a further 40 sections had been designated by 1986, and the enterprise had become an attempt to codify the microbiological literature without a rational basis. Much of the data recorded was of limited longevity. For example 3 large sections (19, 35 and 40) are devoted to growth inhibition by some arbitrarily chosen antibiotics many of which have lost or will lose all clinical interest. Despite these and other criticisms the database is of great value and is available to the public through the BT Tymnet company (Customer support telephone number - 1-301-881-9020). Access to this source requires a $72 joining fee and thereafter user fees of about



CODE SHEET FOR STRAIN CHARACTERISTICS
SECTION 1 - GENERAL INFORMATION

DATE OF RESPONSE
DAY 01-31
MONTH 01-12
YEAR 00-99

DAY	MO.	YR.	EDITION OF QUESTIONNAIRE USED (From title page)	SURNAME (Family name)	FIRST NAME AND OTHER NAMES AND OR INITIALS (Leave one space between each name.)	TITLE OR RANK (e.g., Professor, Doctor, Mister, Captain etc.)
01	12	84		JONES	FRANCES ELAINE	MICROBIOLOGIST

NAME OF PERSON SUBMITTING INFORMATION

COLLECTION NUMBER IN THE WORLD DIRECTORY OF CULTURE COLLECTIONS (If Assigned)	POSTAL ADDRESS
	NIDR NIH BETHESDA MARYLAND 20205

CATEGORY OF MICROBE BEING DESCRIBED (See code*)	NAME OF GENUS OR ARBITRARY NUMERICAL CODE FOR GROUP OR GROUP NAMES (See below**)	NAME OF SUBGENUS (Insert the name only if data being submitted for strains is arranged under subgenera, otherwise leave blank.)	FOR HOW MANY SUBGENERA OF THE GENUS ARE YOU SUBMITTING DATA	SPECIFIC EPITHET OF TYPE SPECIES FOR THIS GENUS
BT	SALMONELLA			

*MICROBE CODE

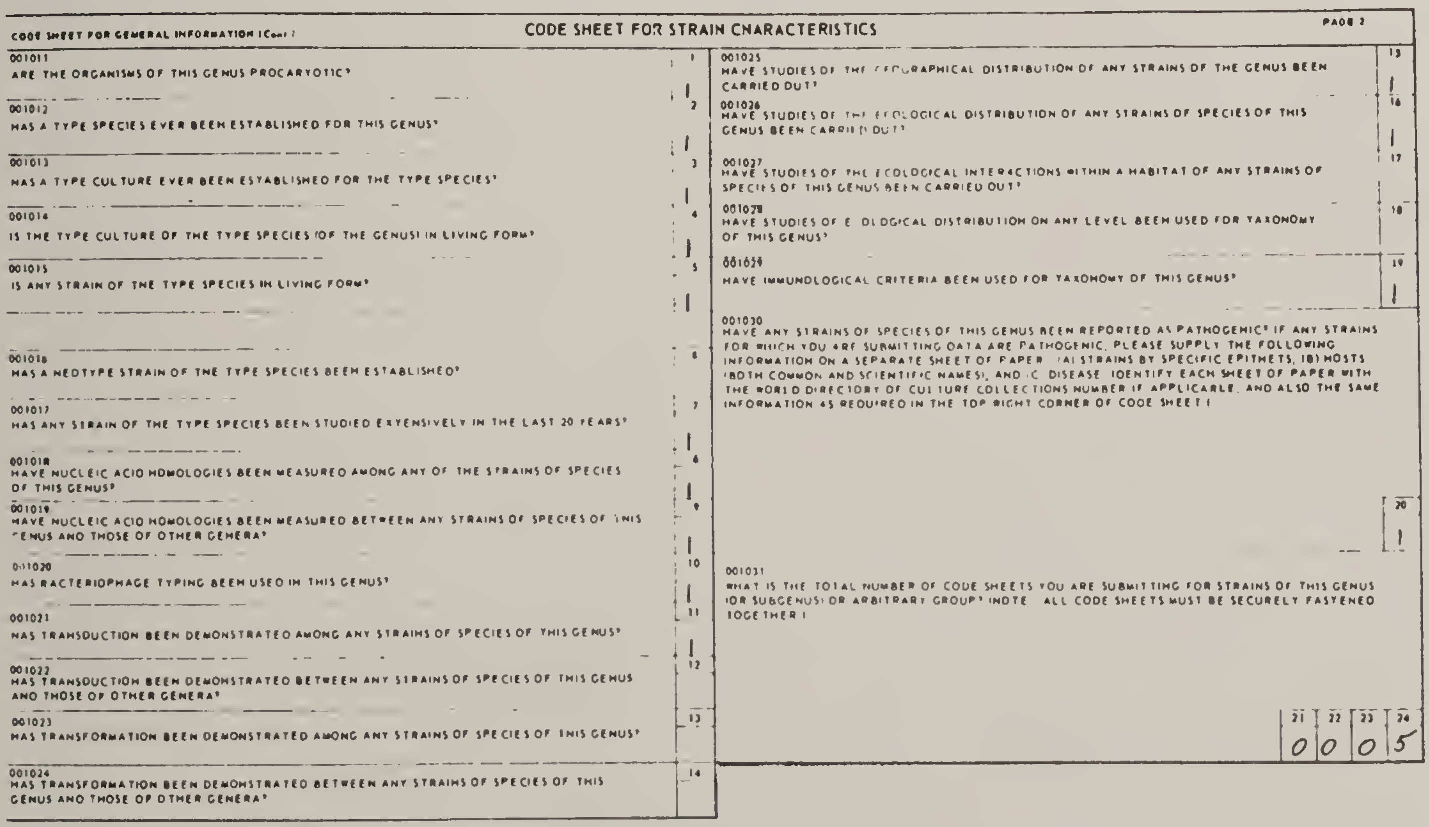
AL ALGAE	BT = BACTERIA	VA = VIRUSES - ANIMAL
FN FUNGI	BR = RICKETTSIA	VB = VIRUSES - BACTERIAL
FY YEASTS	BM = MYCOPLASMA	VI = VIRUSES - INSECT
PZ = PROTOZOA	OO = OTHER	VP = VIRUSES - PLANT

**NOTE 1 COMPLETELY DIFFERENT SETS OF CODE SHEETS MUST BE USED FOR EACH GENUS OR GROUP.
NOTE 2 IF THE DATA BEING SUBMITTED FOR STRAINS IS ARRANGED UNDER SUBGENERA, COMPLETELY DIFFERENT SETS OF CODE SHEETS MUST BE USED FOR EACH SUBGENUS. THE GENERIC NAME MUST BE INSERTED FOR EACH SET.
NOTE 3 IF THE GENUS NAME IS NOT KNOWN AND DATA IS BEING SUBMITTED FOR ANY ARBITRARY GROUP OF ORGANISMS, USE (A) AN ARBITRARY NUMERICAL CODE, E.G., "GROUP 22" OR SIMPLY "22" OR (B) A GROUP NAME, E.G., CORYNEFORMS. SEE DIRECTIONS FOR FURTHER EXPLANATION.

Fig 1 General information Coding Sheet for strain characteristics used in the Rogosa-Krichevsky-Coldwell system

$50 h^{-1} are imposed. There are additional charges for downloading information e.g. by electronic mail and for storage to permit retrieved information to be accessible in subsequent sessions.

The second attempt to construct a microbiological database that will be discussed is that initiated by the biotechnology action programme of the Commission of the European Economic Community. The idea was to construct a network of databases each set of data representing a major culture collection in Europe (possibly restricted



CODE SHEET FOR GENERAL INFORMATION (Cont.)
CODE SHEET FOR STRAIN CHARACTERISTICS
PAGE 2

001011
ARE THE ORGANISMS OF THIS GENUS PROCARYOTIC? 1

001012
HAS A TYPE SPECIES EVER BEEN ESTABLISHED FOR THIS GENUS? 2

001013
HAS A TYPE CULTURE EVER BEEN ESTABLISHED FOR THE TYPE SPECIES? 3

001014
IS THE TYPE CULTURE OF THE TYPE SPECIES (OF THE GENUS) IN LIVING FORM? 4

001015
IS ANY STRAIN OF THE TYPE SPECIES IN LIVING FORM? 5

001016
HAS A NEOTYPE STRAIN OF THE TYPE SPECIES BEEN ESTABLISHED? 6

001017
HAS ANY STRAIN OF THE TYPE SPECIES BEEN STUDIED EXTENSIVELY IN THE LAST 20 YEARS? 7

001018
HAVE NUCLEIC ACID HOMOLOGIES BEEN MEASURED AMONG ANY OF THE STRAINS OF SPECIES OF THIS GENUS? 8

001019
HAVE NUCLEIC ACID HOMOLOGIES BEEN MEASURED BETWEEN ANY STRAINS OF SPECIES OF THIS GENUS AND THOSE OF OTHER GENERA? 9

001020
HAS BACTERIOPHAGE TYPING BEEN USED IN THIS GENUS? 10

001021
HAS TRANSDUCTION BEEN DEMONSTRATED AMONG ANY STRAINS OF SPECIES OF THIS GENUS? 11

001022
HAS TRANSDUCTION BEEN DEMONSTRATED BETWEEN ANY STRAINS OF SPECIES OF THIS GENUS AND THOSE OF OTHER GENERA? 12

001023
HAS TRANSFORMATION BEEN DEMONSTRATED AMONG ANY STRAINS OF SPECIES OF THIS GENUS? 13

001024
HAS TRANSFORMATION BEEN DEMONSTRATED BETWEEN ANY STRAINS OF SPECIES OF THIS GENUS AND THOSE OF OTHER GENERA? 14

001025
HAVE STUDIES OF THE GEOGRAPHICAL DISTRIBUTION OF ANY STRAINS OF THE GENUS BEEN CARRIED OUT? 15

001026
HAVE STUDIES OF THE ECOLOGICAL DISTRIBUTION OF ANY STRAINS OF SPECIES OF THIS GENUS BEEN CARRIED OUT? 16

001027
HAVE STUDIES OF THE ECOLOGICAL INTERACTIONS WITHIN A HABITAT OF ANY STRAINS OF SPECIES OF THIS GENUS BEEN CARRIED OUT? 17

001028
HAVE STUDIES OF ECOLOGICAL DISTRIBUTION ON ANY LEVEL BEEN USED FOR TAXONOMY OF THIS GENUS? 18

001029
HAVE IMMUNOLOGICAL CRITERIA BEEN USED FOR TAXONOMY OF THIS GENUS? 19

001030
HAVE ANY STRAINS OF SPECIES OF THIS GENUS BEEN REPORTED AS PATHOGENIC? IF ANY STRAINS FOR WHICH YOU ARE SUBMITTING DATA ARE PATHOGENIC, PLEASE SUPPLY THE FOLLOWING INFORMATION ON A SEPARATE SHEET OF PAPER (A) STRAINS BY SPECIFIC EPITHETS, (B) HOSTS (BOTH COMMON AND SCIENTIFIC NAMES), AND (C) DISEASE. IDENTIFY EACH SHEET OF PAPER WITH THE WORLD DIRECTORY OF CULTURE COLLECTIONS NUMBER IF APPLICABLE, AND ALSO THE SAME INFORMATION AS REQUIRED IN THE TOP RIGHT CORNER OF CODE SHEET 1 20

001031
WHAT IS THE TOTAL NUMBER OF CODE SHEETS YOU ARE SUBMITTING FOR STRAINS OF THIS GENUS (OR SUBGENUS) OR ARBITRARY GROUP? (NOTE: ALL CODE SHEETS MUST BE SECURELY FASTENED TOGETHER.)

21	22	23	24
0	0	0	5

Fig 2 Detail of code sheet for strain characteristics (Rogosa et al., 1986)

to one node on the network for each country). The data in each set were to have the same format and were to be accessible by the common BASIS utility (Information Dimensions Inc., Dublin, Ohio, U.S.A.) . The database included large collections in the Netherlands, Belgium and the United Kingdom. The format for data entry has been published (Gams et al., 1988) and the system named Microbial Information Network Europe is planned to be on-line in 1992, but at present the International Mycological Institute's data and that of the National (U.K.) Yeast collection are available through the BT Tymnet company. The format is defined in a PASCAL sense of 3 record types within a main data record - each record then being subdivided into fields, 114 of which are described in the publication (Gams et al. 1988). These fields may or may not be duplicated in the record types. The fields are grouped into blocks as shown in Table I, presumably to aid in the entry of data. The data is also schismatic because 84 fields are not universally available on the network. Eight of these, grouped in a block called 'internal administration' contain such essential data as the accession number of the organism and the dates of acquisition and update. Obviously some of this information (though not all; Table I) has to be duplicated in the fields of the records that are available to the public. Seven fields dealing with references to metabolites, ecological, pathological, taxonomic (duplicated) genetic and patent literature will also be, surprisingly, unavailable to network users at the onset. Table I gives a cursory indication of the information stored in the various records and it will be seen that although it is less comprehensive than that envisaged by Colwell and her coworkers (Rogosa et al., 1986), it is, nevertheless, voluminous.

It seems likely that these two databases will provide microbiologists with very useful and important services in the future. One hopes that the relatively large funds needed for their maintenance and development will be available, but even if they are, these

Table I Data blocks used in the Microbial Information Network, Europe data bases

Block description	Number of Fields	Fields available on network	Notes
Administration	8	0	
Species name	13	7	Serotype & taxonomic data not on network
Strain administration	11	5	Accession number given; date of receipt not available
Authenticity of specimen	1	1	
History of isolate	14	7	Herbarium specimen numbers not on the network
Symbiosis, parasitism toxicology etc.	8	1	Only information on pathogenicity on network
Sexual state & behaviour	2	2	
Histology & cytology	10	0	
Genetics	12	2	Mutants & 'killer' yeasts information only available
Growth requirements	23	2	Only conditions for growth (incl. T factors) on solid media available on network
Enzymology, metabolites etc.	6	0	
Practical applications	6	3	Industrial applications included in 2 records - assay & patented organisms.

databases have several deficiencies from the point of view of the working scientist. Among these shortcomings are the impossibility of foreseeing the needs of future microbiologists, the inevitable expense of consulting the services, and perhaps most important, the difficulty of providing a mechanism of enabling microbiologists to interact and therefore improve the system. These problems are evident in the Chemical Abstracts database which contains many errors and omissions that are apparently immune from correction by working chemists.

About 12 years ago we installed a search and retrieval system that enabled scientists to locate fungal metabolic products reported in the literature (Brewer et al., 1978). The references were the product of more that 30 years reading by two working mycologists. The data were updated regularly, on the basis of our routine perusal of the literature and was supported by the National Research Council. The number of accessions of the system on a yearly basis were as follows: 1981, 18; 1982, 26; 1983, 31; 1984, 109; 1985, 52; 1986, 90 (the figure for 1986 is an estimate subtracting the increased usage after the culture collection database was added to the system, see below) and the average cost (i.e. discounting overheads such as storage charges) of a single search was about 90 cents. The use that was made of these data showed that it was possible to allow public access to a utility on a large main-frame computer at modest cost and without accidental or deliberate corruption of the system. It therefore seemed a worthwhile experiment to attempt to provide a similar system which would give access to information about live cultures held in Canadian culture collections. At the outset the data were restricted to fungi, because it became apparent to a number of mycologists attending a meeting of culture collection curators in London, Ontario in 1986, that a general agreement to establish a national culture collection database was likely to take a long time. Accordingly 10 curators agreed to make their data available and these data form the basis of the system described in this paper.

Methods

Computational equipment Most of the data was originally written using a Texas Instruments terminal equipped with dual casette drives. The data lines were corrected using the limited editing facility of this machine. Later an Apple Macintosh computer with its greater editing capability was used and the data were stored on 3.5 inch discs. All data were transmitted to the mainframe computer via a telephone line using the 'kermit' utility. Thus all crude data have been retained on one or other of these media. The database and its attendant programmes were originally implemented on a Control Data Cyber 170 machine with the NOS operating system. After about 4 years of use the system was transferred to a Digital VAX 8800 unit with VMS operating system. The VMS procedures giving access to the files are described in this paper; earlier NOS procedure files are available to interested readers on application.

Sources of Collection Data Eleven culture collections took part in this experiment. Ten of these were Canadian and of the other (International Mycological Institute, Kew, U.K.) only about one third of its holdings were incorporated into the database. In Table II the addresses of the collections, the name of the scientist donating the data, an acronym to distinguish the collection in accession numbers, the medium on which the data was submitted, and the numbers of cultures about which data were given, can be found. The data records for the collections at the University of Alberta and at Kew were more extensive than required for this database (see below). The donators of the data of these collections therefore submitted tapes containing the data we required, thus this

Table II Description of sources of culture collection information used in the 1991 database

Title of Collection	Postal Code	Curator or Donator	Acronym	Number of Cultures submitted	on database	Data received on
Natl. Research Council						
Halifax	B3H 3Z1	D. Brewer	HLX	1037	751	Index cards
Ottawa	K1A 0R6	R. Latta	NRC	478	478	Typed list
Saskatoon	*	R. Haskins	PRL	1266	1084	Index cards
Can. Forestry Service						
Edmonton	T6H 3S5	Y. Hiratsuki	NOF	652	652	Catalogue
Fredericton	E3B 5P7	L. Magasi	FSC	642	642	Catalogue
Forintek	K1G 3Z5	K. Seifert	FTK	2060	2245	Catalogue
Laval Univ.	G1K 7P4	R. Fortier	CRBF	380	380	Typed list
Labatt's Brewery Co.	N6A 4M3	I. Russell	LBT	354	353	Typed list
Agriculture Canada	K1A 0C6	S. Needham	DAOM	8329	7923	Notebooks, index cards
Univ. of Alberta	T6G 2E1	L. Sigler	UAMH	5041	5075	Magnetic tape
Commonwealth Mycological Institute (U.K)	TW9 3AF	D. Hawksworth	CMI	~8050	2624	Magnetic tape

*Now incorporated into the Agriculture Canada collection

editing of the information may well have introduced some of the errors we ultimately detected. All the information submitted was checked for mycological accuracy, for compatibility with published data on appropriate isolates and for internal discrepancies. In most cases these problems were resolved by correspondence with the curators and/or the mycologists who originally isolated the fungi. In certain cases accession numbers had to be changed. A list of these numbers, the new numbers assigned and the reasons for the changes are available upon enquiry by curators.

Data Format In selecting a format for the data to be recorded for each isolate, we were greatly influenced by the arrangement of the data records devised by Martin and his colleagues (Martin, 1964) because it appeared to be inexcusable to promote a format into which all previous work could not be included. We therefore adopted the concept of one record for each isolate, and subdivided this into 4 fields, 3 of which (the organism binomial name, its accession number and the substrate from which it was isolated) were identical to those used by Martin (1964). The fourth field contained information on the source, maintenance (i.e. culture and/or storage conditions) and the date of accession of the organism to the collection as in Martin's scheme. However, because the number of methods of storage of fungi are small and the sources of fungi, i.e. own isolation or a culture collection, relatively small, this information was stored numerically, and decoded by incorporating the translation in the search programme or in a suitably indexed file. Thus this fourth field was a multidigit number, the justification of which permitted retrieval of the information in a comprehensible way. For example, 20331984 indicated on translation that the culture was acquired in 1984 (4 right digits), was stored under oil and regularly subcultivated (33=100001 binary; each digit in the binary number corresponding to one method of maintenance) and that it was originally obtained from a culture collection in the file of sources whose index is 20 (the two left justified digits). As Colwell and her colleagues (Rogosa et al., 1986) have pointed out, this technique is to some extent open ended, since the number 20331984 can be extended (left justified) to the limit imposed by the computer's arithmetical processing capability. We have used this possibility by adding to the data information on the availability of the organism i.e. whether the collection charges a fee for supplying a culture or whether it is freely available, the pathogenicity of the organism, and a cross reference to the file of fungal metabolic products described in the introduction to this paper. This information could be added as two further left justified digits e.g. 1220331984, the most significant digit (1) indicating a reference on the 'mycotox' file and the next (2) indicating that the culture could be obtained for a fee.

Computer programme development

Assembly and Updating of Database Files The database was written using the beginners all-purpose symbolic instruction code (BASIC). The main reason for choosing this language was that each organism in a collection could be assigned one data statement on one line of text - each line being numbered. It was found that this format simplified checking for typing errors, spelling errors in the original documents, omission of data, computational errors and editing procedures e.g. replacement of common names with binomial names in the substrate field. The database was assembled in blocks of about 500 organisms and was written in two ways. The first was stored in binomial format and was indexed with respect to organism name and accession number. The second was written in readable text, each culture collection being a separate subfile. The format of both was the same and was as follows. Each record was limited to 105 characters (bytes) divided into the following fields: 0-13, accession number; 14-26, the numerical code described above; 27-57, the substrate description and 58-104 the binomial name.

The indexed file was used for public access i.e. for search and retrieval purposes and was recreated from time to time as changes in the collections occurred. These changes were made in the second file and could be cumulative i.e. changes could be made as received, and then at regular intervals a new indexed binomial file written for public use. Much of this editing was done on the Macintosh computer which was especially helpful for updating the literature cross references and compiling statistics of the database such as those reported in this paper.

Programmes to Search and Retrieve Information from the Database Information is retrieved from the database by a user employing a computer programme written in the common business-orientated language (COBOL). This programme was written in such a way that versions of it could be called by a user in any language that uses Greco-Roman script, with the restriction that input and output statements had to fit the defined picture formats. The source text of the programme was broken down into a number of files, one being the code that retrieved and formatted the information required. This information was obtained from three sources: 1, the main database; 2, a file of names and addresses of culture collections and 3, files containing on-line help (see below). In addition (in this particular implementation) there were two files that contained definitions of all programme prompts and messages (one in English and one in French). This programming architecture is shown in Fig 3. The 'logical name' facility of the VAX(Digital)/VMS operating system was used to build the executable programmes. The COBOL 'copy' statement shown in Fig 3 refers to the language file by the name

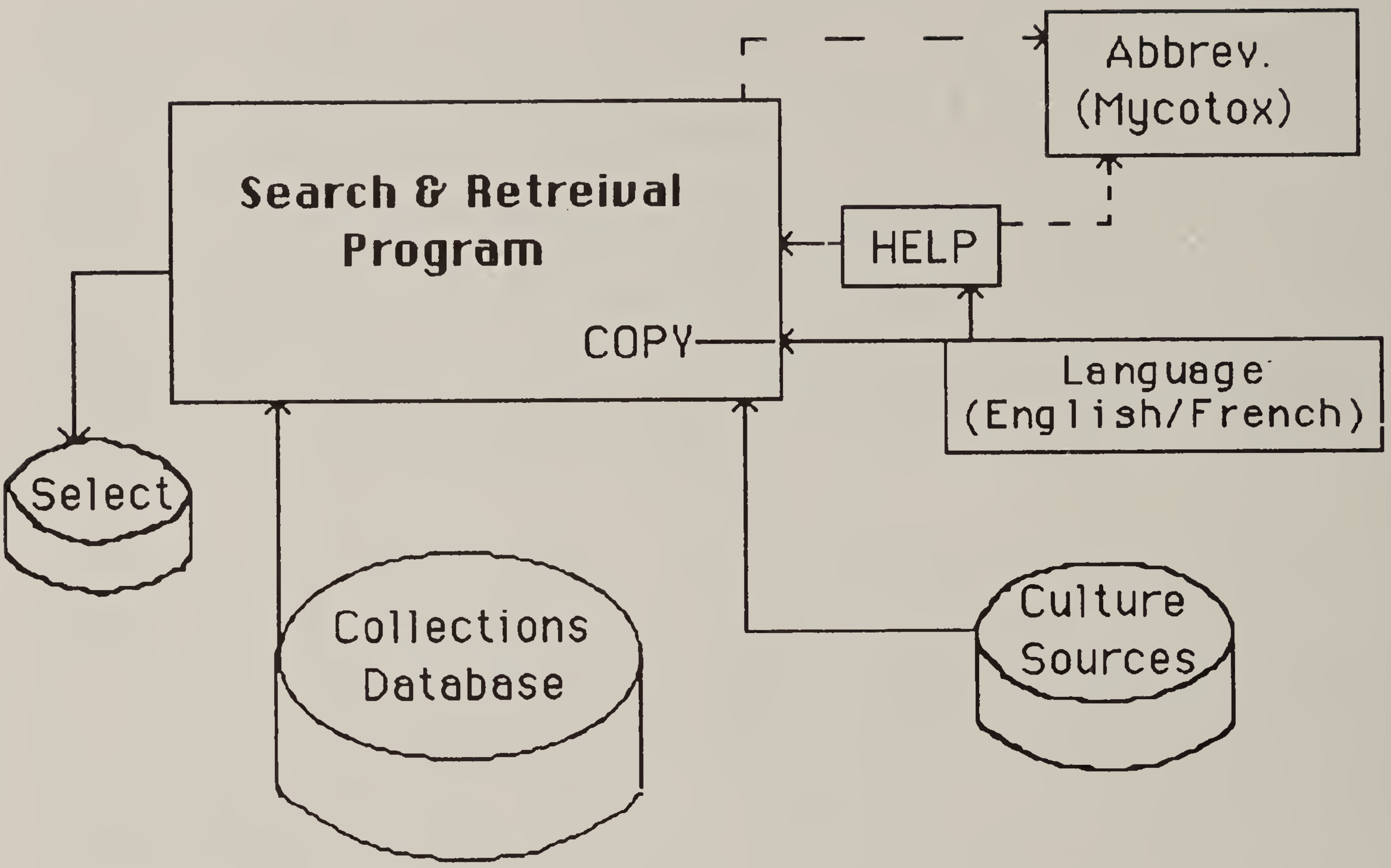


Fig 3 Files used by the programme for retrieval of information from the culture collection database. These files are called by the procedures 1, 2, & 3 in Fig 4.

'query–language'. Before passing the main source file through the COBOL compiler a VMS logical name is defined, thus allowing an English or French language file to be selected without changing the text of the main programme. The same technique is used to select a help file in the language of the user's choice. Hence there are separate executable query programs for each language of the system, each being assembled by defining the query language, compiling the COBOL main programme and linking the resulting object file to make the (English/French) executable program.

Two other features of the programming need to be mentioned. First it was desired to impose a limit on the output data because many cultures of the same species were maintained in some, or even most collections. Thus an enquiry for such a common species resulted in the output of several hundred lines of data. Output was controlled by writing this data to a scratch file before displaying anything to the user. The user was placed in control of how much of the data from the scratch file would be printed and/or displayed on the terminal screen. Thus the full data for one culture could be displayed or abbreviated details: binomial name, accession number, and the substrate on which it had been found for each of 10 organisms – could be printed. A subroutine was written to create a pause at this point giving the user the following choices - 1, a list of a further 10 organisms in the abbreviated format; 2, display of the full data for one organism by typing its accession number, and 3, to terminate the enquiry and/or relist the data. Help was offered to the user at this point by displaying the number of cultures remaining on the scratch file.

The second feature of the programming that requires comment relates to searching the database for organisms isolated from particular substrates. The term 'substrate' was used loosely and this part of the record contained a variety of information e.g. the binomial name of the plant on which the organism was found, the approximate geographical location of the isolation, characteristic enzymology etc. Code was therefore written to 'inspect' the substrate field for the occurrence of a string input by the user. Only when this string was found in the substrate field of the organism selected, was the data recorded on the scratch file.

Security The culture collection database and the list of references to mycotoxins are intended to be available to everybody. Though they contain no confidential information there remains the danger that the data and/or the computer system may be destroyed or corrupted deliberately or unintentionally by a user. It is therefore necessary to reduce such possibilities to a minimum.

Computer System Security In the VAX(Digital)/VMS system each valid username has a record in the system's 'authorization file' that contains e.g. the user's password, the number of central processor units that may be used etc. It is possible to invoke an unusual configuration where no password is required. This simplifies the login procedure for the user and discourages password exploration. The user's record in the authorization file also contains the specification of a command file (login.com) which is normally executed after the computer has checked the validity of the username and password but before access to the system is granted. In the present instance, this login.com file has been carefully written to try to ensure that a user cannot induce it to completion and thus gain access to all the computer facilities. The login.com file presents the user with a limited number of choices. One of the choices logs off the user, thus terminating the login.com procedure file (instead of gaining access to the computer system as a whole). The remaining choices in the login.com file give the user access to the culture collection database or the list of references to mycotoxins. An incorrect choice merely results in the menu of choices being repeated. This procedure is written as a loop so that the session is not terminated after a search but again presents the user with the menu of legal choices. In addition to the precautions taken in the login.com procedure, the username used for searching the database is flagged in the authorization file as "capture" which means that access to the system other than under the control of the login.com procedure is not allowed, thus protecting against errors in the login.com procedure. The procedures described are illustrated in the flow-chart of Fig 4.

Database Security The databases represent many hours of work in their assembly and the checks that have been made for accuracy. In addition, the curators who have supplied us with the data presumably own copyright to their segments of the data, which therefore have to be protected. We have sought to achieve this protection by the following methods. The datafiles are regularly copied to magnetic tapes, by ourselves and by the backup system of the computer. One tape is always kept outside the computer center. However, this does not exclude unauthorized or inadvertent changes that do not change the data format but which are cumulative and difficult to detect (gross changes would result in input/output errors during execution of updating programmes). To prevent user tampering with the files the username used to log onto the search programmes was given no library of files i.e. the databases and the programmes that search them were not located in the directory of that user number. Instead, permission was given for the user to read these, and only these, files that resided in the library (directory) of another user. This second user was conveniently ourselves, since this permitted us to update the files, improve the programming and exclude our use of the files from the outside use of the system. It also permitted two copies of the databases to be kept, one of which was not used by the public and hence comparison of the two files provided a further check on the integrity of the data.

Instructions for searching the databases, when on line are given in Appendix A.

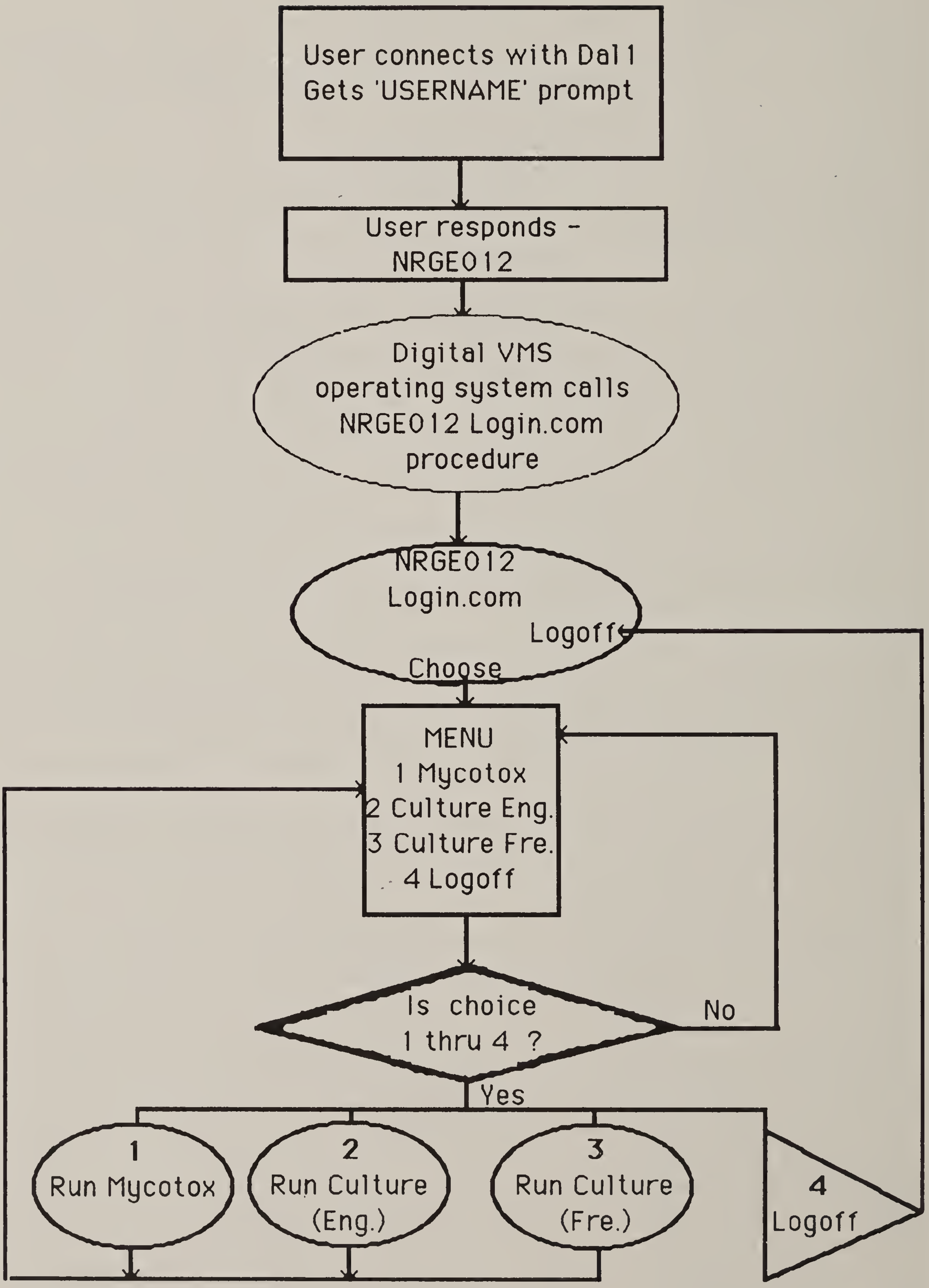


Fig 4 Execution flow-sheet of the Login.com file associated with the user number for public access of the database. Note that this Login.com file never proceeds to completion.

Results

Expenses incurred in setting up the databases

Literature reference file ('mycotox') This file and the programmes that searched it were set up in 1978 and the cost for doing this was $7426 broken down as follows: secretarial assistance $5567, computer processing $1667 and publication $192. The cost of writing the search and retrieval programmes was paid by Dalhousie University Computer Center because they were interested in the project from the point of view of open access to the computer services.

Culture collection database This file and the programmes that retrieved data from it were set up in 1986 at a total cost of $33598, broken down as follows: secretarial assistance $25662, contracts awarded to the University of Alberta ($500) to provide us with data and Dalhousie University ($2000) to write search & retrieval programmes, computer processing charges ($5111) and the expenses of demonstrations, correspondence etc.,$325. To this must be added part of the capital cost of the computer terminals used; this is deferred as they were also used in the maintenance of the databases.

Costs of maintenance of the databases

The disc storage charges for the literature reference file and its ancillary programmes in the period 1979-1985 inclusive were $505±40 y^{-1}. These storage charges increased after the culture collection database was added; the mean cost for the years 1987, 1988 and 1989 was $2505 y^{-1}. Another major cost of maintenance was that of updating the system and of correction of the data on the files. The system was updated when it was transferred to the VAX 8800 computer system and this was done by contract to Dalhousie University ($6000). The data on the literature reference file were corrected - usually by adding new references as they were published - on a weekly basis and the backup tapes rewritten monthly, the mean cost of these activities in the period 1979-1987 was about $90 y^{-1}. After the culture collection data had been added this expense increased to $2112 in 1988 and $1549 in 1989. A major item in this increased expenditure was the update of the cross references in the culture collection database to cover new work that had appeared in the preceding year.

Finally there is the capital costs of terminals $2900(=50%), tapes $75, floppy discs $150 etc. thus the total for setting up and maintaining the system was $65670.

Users and cost of usage of the databases

The number of users of the mycotoxin literature references file in the period 1981-1986 are given in the introduction of this paper. These numbers increased after the culture collection data was added as follows (year, number of accessions, total connect time in hours): 1986, 106, 5.63; 1987, 137, 9.699; 1988, 124, 5.111; 1989, ?, 6.677. The large number of connect hours in 1987 is probably due to curators of the collections learning how to use the system. The number of accessions to the system in 1989 is not known because the VAX accounting algorithm does not report this statistic. However, it is clear from the increases in central processing units used and connect time, that there was greater use of the system in 1989. The total cost of this activity was $1688 and on a yearly basis (rounded to the nearest dollar) 1981, $21; 1982, $30; 1983, $36; 1984, $120; 1985, $50; 1986, $295; 1987, $397; 1988, $232; and 1989, $507. Thus the total cost of the system over the period 1979-1989 was about $70,000. More significantly, perhaps, the running costs (allowing for amortization of capital costs and major system updates over 10 years) per annum are approximately $6000. None of these costs include the time and knowledge of two mycologists who were primarily responsible for setting up the system and maintaining it. The value of this work is difficult to compute because it was in part voluntary because of their scientific interests.

Statistics of the databases

At the end of 1989 there were 19585 records of fungal cultures from 10 Canadian collections and 2624 cultures from the collection of the Commonwealth Mycological Institute, on the database making 22209 altogether. There were 1482 genera of fungi represented in this data; the 30 most common, together with the numbers of isolates of each, are given in Table III. Eleven more genera were represented by more than 100 isolates; there were 53 genera with 50-99 isolates and 242 genera with 10-49 isolates. However the number of single species i.e. genera represented by only one culture was 525 or 35.4% of the total and 200 genera were represented by only 2 isolates. The maintenance of these 19585 organisms is summarized in Table IV. The oldest culture *Phellinus robinae*, was placed in the Agriculture Canada collection in 1912 and the numbers accumulated in the following decades were: 1912-1920, 32; 1930, 261; 1940, 555; 1950, 1428; 1960, 3320; 1970, 4882; 1980, 4492; and 1981-1986 inclusive 2945. The date of acquisition of the remaining 1670 cultures is not known.

Table III Number of isolates of the thirty most common genera on the database

Order	Genus	No. of isolates	Order	Genus	No. of isolates	Order	Genus	No. of isolates
1	*Trichoderma*	755	11	*Polyporus*	296	21	*Verticillium*	148
2	*Penicillium*	726	12	*Candida*	266	22	*Pleurotus*	144
3	*Fusarium*	638	13	*Ceratocystis*	257	23	*Acremonium*	144
4	*Aspergillus*	526	14	*Tyromyces*	245	24	*Paecilomyces*	141
5	*Saccharomyces*	475	15	*Phialophora*	244	25	*Coriolus*	139
6	*Chrysosporium*	455	16	*Arthroderma*	241	26	*Scytalidium*	138
7	*Poria*	389	17	*Suillus*	225	27	*Scopulariopsis*	127
8	*Trichophyton*	367	18	*Phoma*	198	28	*Malbranchia*	124
9	*Chaetomium*	354	19	*Fomes*	191	29	*Pythium*	123
10	*Peniophora*	348	20	*Phellinus*	184	30	*Alternaria*	123

Table IV Numbers of isolates in Canadian collections maintained by different methods and kept as herbarium specimens

Method of maintenance	Number of isolates
By regular subcultivation	8849
refrigeration at 4°	5733
lyophilization	9915
refrigeration at -196° (i.e. under liquid nitrogen)	479
under mineral oil	8497
Herbarium specimens	7339

Table V Canadian Provincial and Territorial distribution of isolates recorded in the database

Province/Territory	Number of Isolates
Alberta	2541
British Columbia	1148
Manitoba	243
New Brunswick	322
Newfoundland (including 5 from Labrador)	60
Nova Scotia	488
North West Territories (including 19 from Baffin Island)	50
Ontario	2817
Prince Edward Island	65
Quebec	1346
Saskatchewan	239
Yukon	18

Almost half (47.7%) of the cultures (9338) were isolated from Canadian sources, distributed among the Provinces and Territories as shown in Table V. The relatively large number from Alberta reflects the fact that two of the collections were from that Province, in one of which about 20% of its holdings were species of Arthroderma, Trichophyton and Chrysosporium (? human pathogens). Forty percent of the cultures (7874) in the database were isolated by the curators of the collections or by their colleagues, but 8553 (43.7%) were of unknown origin. A relatively small proportion (2442, 12.5%) were isolated from soil samples. Only 1243 (6.4%) were bought from the American Type Culture Collection (375), the International Mycological Institute (196) or the Centraalbureau voor Schimmelcultures (672).

About 20% of the isolates (4221) on the culture collection database were species that have been reported to produce toxic metabolites. The file of literature references contained 3382 entries at the end of 1989 in which 1155 species were described. It must be emphasized that the identity of a species in the culture collection database with one reported in the literature to produce toxic metabolites does not imply that the former produces the antibiotic(s) in culture.

Downloading and storage

The programme and procedure files, some 18 in all, used for setting up the system described in this paper have been copied to 3.5" (8.9 cm) magnetic discs in both the Macintosh Apple format and the International Business Machines (IBM) format i.e. they can be read on either computer with the aid of a rudimentary word processor. These discs have been deposited and catalogued in the library of the Nova Scotian Institute of Science. Copies of them may be bought from the Librarian of the Institute. The files can be loaded via a utility such as 'Kermit' onto any Digital VAX computer using the VMS operating system and having a COBOL compiler. Help will be required from the operators of such a computer system to set up the appropriate authorization code. For other main frame computers, a COBOL compiler for the source code will be required but given the files (copies of these are on the discs) called by the main programme there should be little difficulty in linking them and setting up an executable program.

The 'mycotox' literature reference file has also been deposited in the library of the Nova Scotian Institute of Science, both on 3.5" magnetic discs in either Macintosh or IBM format and also on 9-track magnetic tape. This file is available from the Librarian of the Institute for a fee that will be decided by the Council of the Institute when the expenses of doing this work are known.

The culture collection database file copied on 9-track magnetic tape, has also been deposited in the library of the Institute. It can be consulted freely by members of the Institute at the library provided that they give adequate notice to the Librarian. Copies of the data are only available after written permission has been obtained from the appropriate curators whose names and addresses are given in Table I, and signed copies of this permission are sent to the Librarian of the Institute. It is unnecessary to obtain permission from curators whose data is of no interest to the user, since any or all of the 11 culture collections data can be copied. As in the case of the 'mycotox' file it is the Institute's policy to apply a fee for these services which will be decided when their cost is fully known.

Discussion

Canadian microbiologists were pioneers in the use of computers to store and process information on bacteria, fungi, protozoa and other micro-organisms. In their publications (Martin, 1964; Simpson et al., 1971) they present strong arguments for the mechanical documentation of such information and these reasons remain valid (Russell & Sanderson, 1988). How is it, then, that this considerable body of work is presently unavailable to the public ? There are, of course, several reasons, some of which are worthy of comment. At the time (1964-1970) access to large bodies of data stored on mainframe computers could only be through the intermediary of computer scientists, who were uninterested in the microbiological use of the data, or by microbiologists prepared to learn computer programming. Inevitably the information became otiose and increasingly redundant. In addition, one of the main reasons for creating the database was to explore the feasibility and utility of such systems - their maintenance and longevity were secondary considerations. Thus when the interests of the scientists who created the database changed it was easy to terminate the support required for maintenance and development simply by default. Traditionally, storage of scientific knowledge has been achieved on a long-term basis by scientific societies in their published journals, which were then (and are) accumulated in libraries. These societies have been slow to react to the changes that have occurred in the last 10-15 years in the technology of information dissemination. This prudence was and is based on sound reasons; the tendency of purveyors of information media - the paper tape to compact disc progression - to change the format and hence the machines to read and write information, the high cost of computational machinery and so on. However, in recent years the cost of personal computers has declined to the point where many scientists can afford their own machines and the 3.5 inch magnetic disc medium, capable of recording about 10^6 alphanumeric characters is now available for less than 50¢.

Thus a scientific society has the option, when moving into the new technology, of presenting scientific data to the public in the form of magnetic discs and/or in the form of an online utility accessible on a main-frame computer. These considerations have led us to give a cost analysis for the implementation and maintenance of a small mycological database on line. It is clear from the analysis that the maintenance costs per year, of this database are not trivial, but the fact that they are about the same as one issue of these Proceedings makes their undertaking by a scientific society feasible. As in all other publication activity by scientific societies, the style or format of this new medium will have to be as concise as possible. Thus the format for the culture collection database was chosen not only to conform with that used by preceding workers (Martin, 1964; Simpson et al. 1971), but also, uniquely, to embrace our conception of the minimal essential information required. We see this choice as an advantage for other workers because they will be able to use these data as a basis for their own databases and they will have the minimum inconvenience in updating their segment of the whole.

The financial analysis also provides information on the merits of an online utility as compared to one based on magnetic discs. Thus when 2624 isolates from the collection at Kew (U.K.) were added to the database - an increase of about 12% - the cost of a search of the data (~90¢) remained the same but the storage cost increased by about \$500 y^{-1}. About four times this information could be stored on a 800 Kbyte disc for <50¢. Thus it is conceivable that the distribution of information on discs will compare favourably with on-line facilities. Details of a proposal to implement such a cooperative database are given in Appendix B.

If this happens the role of the scientific society will remain traditional. It will serve to collect, review and publish scientific information in an electronic format as well as by the familiar printed form. Its success or failure in this endeavor will depend, as always, on the support it receives from scientists. The statistics given in this paper show that there is a demand in Canada for mycological information in an electronic format, but the concept of cooperation for a national culture collection database is less secure.

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Appendix A

Instructions for using the Databases

In the following instructions certain conventions are used. The sign ® is an instruction to press the 'enter' or 'return' key; CAPITAL letters are used to indicate the response of the computer and *italic* letters are used to indicate information typed by the user (regardless of the actual case input or output). Some of the commands can be abbreviated e.g. the list command can be either list or simply l. In the following discussion abbreviated commands are printed thus *l)ist.*

Use of the system is similar to that described in the earlier publication (Brewer et al., 1978). One logs onto the system either using a Datapac line (No. = 76100256) or by a telephone line (No. = 01-902-494-2500). When a connection is established - a buzz is heard and the user responds with ®. The following procedure is then followed:

```
WHAT SYSTEM? dal1 ® ® (i.e. return or enter 2 or 3 times)
DALHOUSIE UNIVERSITY  DECSERVER 200 TERMINAL SERVER V3.0(BL33)-LATV5.1
DS0006
LOCAL-010-SESSION1 TO DAL1 ESTABLISHED
USERNAME: nrge012 ®
CHOISSISSEZ (PAR NUMERO) L'APPLICATION SVP
   1. MYCOTOX - MYCOLOGICAL TOXINS
   2. FUNGAL CULTURE COLLECTION DATABASE
   3. BANQUE DE DONNES DES SOUCHES FONGIQUES
   4. LOGOFF (Q ALSO LOGSOFF)
SELECT BY NUMBER OR TYPE 'Q' TO QUIT
```

The user now selects the utility they wish to use by typing *1* or *2* or *3*, or *4* and ®. By typing *1* access is obtained to the file of literature references to fungi that produce a toxic metabolite in culture. The organization of the file and the method of searching it is essentially the same as in 1978. The list of abbreviations that is part of the 'mycotox' utility (Brewer et al., 1978) has been expanded to include abbreviations used in the culture collection database. Thus to interpret abbreviations used in the latter file it is necessary to exit the culture collection search utility (see below) and enter the 'mycotox' utility through the menu selection process.

Functionally, choices 2 & 3 in the above menu differ only in the language used - according to the preference of the user (such language choice in the case of the 'mycotox' utility seems to be unnecessary since the names of the organisms, metabolites and journals are universally understood). For example when *3* is typed in response to the menu followed by ® the computer responds e.g.

```
              BANQUE DE DONNES POUR LA COLLECTION CANADIENNE
                           DES SOUCHES FONGIQUES
                 (COMPILE PAR LE CONSEIL NATIONAL DE RECHERCHES)
POUR OBTENIR DE L'AIDE OU DES RENSEIGNEMENTS VEUILLEZ TAPEZ 'AIDE'
COMMANDE?
```

There are 3 ways to respond to the prompt COMMAND? when searching for information from the database:

1. If the accession number of an isolate is known (by for example from a list of isolates of a selected organism (see below)), typing (e.g.) a)ccess HLX 136 will generate on the terminal screen all the information on the file of the gliotoxin producing organism *Penicillium terlikowskii*, held in the collection at the National Research Council's laboratory in Halifax.

2. The second way to search the database is to use the command l)ist, thus *l)ist Penicillium terlikowskii* results in the output of the total number of cultures of this organism recorded on the database, all of the accession numbers of these organisms and finally, the substrates from which these organisms were isolated.

This information is printed on one line of <80 characters, hence details of many isolates can be revealed in a small amount of space.

As discussed above the information is output in blocks of 10 organisms when the programme pauses and asks the user:

X MORE RECORDS TO COME; TYPE 'END' TO STOP HERE, A BLANK LINE TO CONTINUE, OR AN ACCESSION NUMBER: (X being the remaining isolates waiting to be printed)

In this particular case the user merely types the accession number without a command word e.g *HLX 136*, if *end* is typed the user is returned to the menu of choices.

3. The third way of searching the database enables the enquiry to restrict a species or genus chosen to those found on, or restricted to, a particular substrate. For example if the substrate is recorded as 'roots of Abies rugulosa N.S.' the search can be restricted to those organisms isolated from 'root', or from 'roots of Abies', or from Nova Scotia, and so on. To do this the required substrate is entered *before* the name of the species sought as follows:

```
COMMAND? s)ubstrate root ®
COMMAND? l)ist Penicillium terlikowskii ®
```

and in this example only those isolates of *P. terlikowskii* found on the roots of plants will be printed. One reverts to the default mode, where in the searches the substrate is ignored by the following:

```
COMMAND? s)ubstrate ®
```

The information revealed about each organism is not restricted to its name, accession number and the substrate on which it was found. The following series of commands prints out all the information on the file for each organism:

```
COMMAND? long ®
COMMAND? l)ist Penicillium terlikowskii ®
```

This additional information is as follows:

1. Whether the organism is freely available from the collection where it resides, is available for a fee, or is available only after consultation with the curator.
2. Whether the organism is pathogenic to man.
3. From where the organism was obtained by the collection - where this is not known, UNKNOWN is printed. If the organism was obtained from another culture collection, the name and address of this collection is displayed.
4. Methods of maintenance of the organism - by one or more of the following methods: subcultivation, refrigeration at 4° or -196° (i.e. under liquid nitrogen), under paraffin oil and/or lyophilized.
5. Whether an herbarium specimen is kept.
6. The year the collection obtained the isolate.
7. Whether or not there is a literature reference to an isolate of this species producing a toxic metabolite.

Thus this degree of detail can be obtained either via an accession number or by a general (l)ist) enquiry for genus or species in the 'long' format. It is important to appreciate that internally the computer writes the full data to a scratch file. Thus segments or all of this scratch file can be printed in either or both formats by use of the 'relist','short' or 'long' (see below) commands; no further searches are performed - the choices are entirely in the hands of the user.

In the cases of organisms that are laboratory isolates, genetically selected and/or the products of molecular genetic manipulation, the substrate field is used for information on genetic characterization - usually the presence (+) or absence (-) of enzymic markers. These are given in accepted biochemical abbreviations using the 3-letter code for amino acids (e.g. ala=alanine) and sugars (e.g. Glc=glucose). Thus the database can be searched for organisms with or without such characteristics. The substrate field is also used to indicate (when known) the status of the organism as an ecto or endo symbiont.

When it is necessary to decipher one or more abbreviations, or to obtain literature references to metabolites of fungi found in the database the following sequence gains access to this information.

```
COMMAND? end ®
```

when the menu of choices is displayed by the computer

```
   1 ®
                          *** NRC ATLANTIC REGIONAL LABORATORY ***
                                  FUNGAL TOXIN REFERENCES
FOR HELP OR INFORMATION PLEASE TYPE "HELP"
COMMAND? l)ist Penicillium terlikowskii ®
PENICILLIUM TERLIKOWSKII              NRRL A-537-A
   GLIOTOXIN ACETYLGLIOTOXIN                JACS 75 2110
PENICILLIUM TERLIKOWSKII              HLX 136
   GLIOTOXIN DEHYDROGLIOTOXIN               J 1966 1799
PENICILLIUM TERLIKOWSKII              O
   BIS-DETHIODIMETHYLTHIOGLIOTOXIN              CPB 34 340
PENICILLIUM TERLIKOWSKII              O
   GLIOTOXIN                                CPB 34 340
COMMAND? a)bbrev NRRL ®
NRRL
   U.S. DEPARTMENT OF AGRICULTURE,  NORTHERN RESEARCH SERVICE, 1815 NORTH
UNIVERSITY STREET, PEORIA, ILLINOIS, 61604, U.S.A.
COMMAND? a CPB ®
CPB
   CHEMICAL AND PHARMACEUTICAL BULLETIN
COMMAND? end ®
```

when the menu of choices is displayed by the computer.

It will be noticed that a few minor changes have been implemented in the 'mycotox' file since the preceding publication (Brewer et al. 1978). These changes result from rewriting the utility in COBOL to improve the response time and to use the powerful editing and search facilities in the Digital VMS software.

Users of the system can obtain help for their searches of the culture collection and the mycotox files on line. We have tried to make this help self explanatory and to use the same commands to give the same results on each file. The following illustrates the use of this on line help:

```
COMMAND? help ®
   FOR HELP ABOUT A PARTICULAR TOPIC TYPE 'HELP XXX' WHERE 'XXX' IS THE NAME OF THE
TOPIC
   FOR A LIST OF TOPICS FOR WHICH HELP IS AVAILABLE TYPE 'HELP INDEX'
   FOR A SUMMARY OF COMMANDS TYPE 'HELP COMMANDS'
TYPE "END" IF THIS IS ENOUGH HELP? help commands ®
COMMANDS
   COMMANDS ARE ENTERED IN RESPONSE TO THE COMMAND? PROMPT.
   COMMAND ABBREVIATIONS ARE SHOWN IN PARENTHESIS.
   ACCESSION     (A) - LIST THE WHOLE RECORD FOR A PARTICULAR ACCESSION NUMBER.
   END               - LOGOFF THE SYSTEM.
   HELP          (?) - REQUEST HELP.
   LIMIT             - SET THE NUMBER OF RECORDS PRINTED BETWEEN PAUSES.
   LIST          (L) - LIST RECORDS FOR A SINGLE GENUS OR SPECIES.
   LONG              - LIST RECORDS IN THE "LONG" FORMAT.
   RELIST        (R) - RELIST THE OUTPUT IN LONG OR SHORT FORMAT.
   SHORT             - LIST ONLY NAME, ACCESSION NUMBER AND SUBSTRATE.
   SUBSTRATE     (S) - SELECT ONLY RECORDS WITH THE DESIGNATED SUBSTRATE.
TYPE "END" IF THIS IS ENOUGH HELP? end ®
COMMAND? end ®
```

when the menu of choices is displayed by the computer.

To logoff from the system completely the user responds to the menu by typing *4* ® when the computer responds:

```
ACCOUNTING INFORMATION
BUFFERED I/O COUNT        XXXXX          PEAK WORKING SET SIZE     XXXX
DIRECT I/O COUNT          XXXXX          PEAK PAGE FILE SIZE       XXXX
PAGE FAULTS               XXXXX          MOUNTED VOLUMES           XXXX
CHARGE CPU TIME           XX secs        ELAPSED TIME              h. m. s
LOCAL-011-SESSION1 DISCONNECTED FROM DAL1
```

This information is worth noting so that telephone or Datapac charges can be checked.

Appendix B

A Proposal to Establish a Cooperative Canadian Culture Collection Database

The format used to describe a culture in a collection in the database reported in this paper requires a record of length 105 bytes. In the Directory of Canadian culture collections (Weldon et al., 1986) the curators of 140 collections reported their holdings. A summation of these holdings indicates that about 120,000 organisms are maintained in the collections. Thus this information could be recorded in 1.26 x 10^7 bytes. In practice the recording medium required is less than this because 47 bytes are allocated for the binomial name of the organism when usually less than 30 are used. For example the data describing the 7923 cultures in the DAOM collection occupy 6.06 x 10^5 bytes, or about 77 bytes each. Thus all the cultures in the directory (Weldon et al., 1986) could be recorded on fifteen, 800 Kbytes, 3.5" magnetic discs, or 3 of the newer 4 Mbytes discs. The cost of such media is about $4.00 and since it can be quickly and easily reproduced in a number of disc formats, this technology offers an attractive way of assembling a national culture collection database as an alternative to the conventional on-line facility described in Appendix A.

The following is an outline of how such a system might operate.

Input of information into a national culture collection database

It is now possible to buy a reconditioned computer for less than $300 so we assume that in the future all scientists will have one. This being the case we assume that the scientist will keep records of his/her cultures on this sort of equipment and because of the biological characteristics of *Homo sapiens*, that the data and the data format used by each scientist will be different. Our hypothetical microbiologist will, from time to time update their data and we propose that when this is done the machine automatically write 2 files of the data. The first will be in the format used by the scientist and the second will involve editing the information to format it as described in this paper. This second file can then be copied to a 3.5" disc. The programming for this operation is easy and to some extent can be standardized. The disc can then be mailed to a central repository. Discussion of the latter is deferred for the moment.

Output of information from a national database

We envisage three kinds of output normally, but not restricted to, the central repository.

1. *News of and about the information available* This will be provided by publication in the Proceedings of the Nova Scotian Institute of Science, and updates will also be published in this journal. Information will gradually accumulate and be revised from time to time and all of it will be available from the Librarian of the Institute in the 3.5" disc format at a modest fee.
2. *Specific queries* By specific queries we mean straightforward questions designed to elicit information from the database e.g. Who has a culture of *Fusarium graminearum* that produces deoxynivalenol ? Such queries can be made to the Librarian of the Institute by mail using a 3.5" disc and the reply sent, again at reasonable cost on the same disc. In this way scientists will be able to build their own culture sources file in whatever format they choose. Requests for statistics can be treated in the same way. We have some evidence that users of the system in the last four years have done this sort of work.

3. *Downloading* Here we are talking about fairly large blocks of data desired for use by e.g. curators who by misadventure have lost their files, or by biologists interested in taxonomic studies of a group of organisms. Such copies of blocks of data may infringe the intellectual property rights of the owners of the data. In these cases the procedure described in the paper is proposed. The minimum nature of the information on each organism will ease its elaboration by the recipient scientist in any desired format.

Central repository

It is proposed that the repository and clearing house for the national culture collection database be a scientific society such as the Nova Scotian Institute of Science. We believe that a dynamic national catalogue of culture collections held in Canada can be developed on these lines; its future will depend, as always, on the cooperation of the scientists who will use it in the course of their studies. They normally document their results by publication in a suitable journal, hence the proposal can be regarded as an extension of this familiar process. The discs they deposit with the Institute of Science will not therefore, differ materially from any other scientific publication.

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COMPARATIVE PRODUCTION OF *Salmo salar* (ATLANTIC SALMON) AND *Salmo trutta* (BROWN TROUT) IN A SMALL NOVA SCOTIA STREAM

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The production rate of juvenile Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) was estimated at a site in Mountain Brook, a small tributary of River Philip, Nova Scotia. *S. salar* were usually more abundant than *S. trutta*, but growth rate of *S. trutta* was significantly greater. For both species, seasonal growth was restricted to a six month period (May to October). For nine sampling dates between June 1986 and June 1988 total salmonid biomass averaged 3.6 g m^{-2} (range 1.2-5.6 g m^{-2}). Production rate was similar for both species; total production was 3.5 g m^{-2} y^{1} from June 1986 to June 1987 and 5.9 g m^{-2} y^{-1} from June 1987 to June 1988. Although *S. trutta* are not native to eastern Canada, a naturalized population has become established in River Philip, and they are cohabiting successfully with native *S. salar.* Future studies on population interactions and possible range extensions of introduced *S. trutta* would be beneficial for the management of salmonids in eastern Canada.

Le taux de production du jeune saumon atlantique (*Salmo salar*) et de la truite brune (*Salmo trutta*) a été estimé relatif à un lieu sur Mountain brook, un petit affluent de River Philip, Nouvelle-Ecosse. *S. salar* s'est ordinairment démontré plus nombreux que *S. trutta*, mais le taux de croissance de *S. trutta* est significativement plus élevé. Les deux espèces laissent voir une croissance saisonnière restreinte à une période de six mois (de mai à octobre). Neuf dates d'échantillonage entre juin 1986 et juin 1988 ont décelé une biomasse totale, en moyenne, de 3.6 g m^{-2} (avec une distribution de 1.2 à 5.6 g m^{-2}). Les taux de production étaient semblables quant aux deux espèces; la production totale s'élevait à 3.5 g m^{-2} pendant l'année juin 1986 à juin 1987, et à 5.9 g m^{-2} de juin 1987 à juin 1988. Quoique *S. trutta* ne soit pas natif du Canada de l'est, cette population naturalisée s'est évidement bien établie dans la River Philip, et elle cohabite avec succès à côté du *S. salar* natif. Des études ultérieures des interactions entre les populations, et des prolongements possibles de superficie occupée par la truite brune introduite bénificiraient la gestion des salmonides au Canada de l'est.

Introduction

Brown trout (*Salmo trutta*) are not native to eastern Canada but hatchery stocking of this species has occurred throughout the maritime provinces (MacCrimmon & Marshall 1968) and naturalized populations have become established in Newfoundland and Nova Scotia. Except for a study on the Avalon Peninsula of Newfoundland (Gibson & Cunjak 1986), there have been few studies describing the population traits of introduced *S. trutta* where they live sympatrically with native brook trout (*Salvelinus*

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fontinalis) and Atlantic salmon (*Salmo salar*) in eastern Canada. In this note, we compare the seasonal growth, biomass, and annual production rate of naturalized *S. trutta* with native *S. salar* and *Salvelinus fontinalis* in a localized riffle habitat in River Philip, Nova Scotia. Estimates of production from this site were of interest because they have not been estimated in streams cohabited by *S. salar* and *S. trutta* in eastern Canada. Freshwater production rates by salmonid populations are greater where species live sympatrically rather than allopatrically (Chapman 1978; Kennedy & Strange 1986). Because of the presence of three salmonid species living sympatrically in River Philip, and because of its southern location, studies at this site might be expected to indicate relatively high or possibly maximum production rates of fish for rivers flowing into the Gulf of St. Lawrence.

Methods

Study site. Juvenile *S. salar* and *S. trutta* were monitored at a small site (about 200m^2) in Mountain Brook, a small second order tributary of River Philip, Nova Scotia. River Philip is a small watershed (about 98 ha of rearing area) which flows northeast and empties into Northumberland Strait (45°50'N, 63°40'W). Initially, the Mountain Brook site was established as part of an extensive study on geographic variation in the morphology of juvenile *S. salar* (R. Claytor, in progress); observed densities of *S. salar* and *S. trutta* at the site, however, indicated the potential for comparing life history traits of both species. The site was predominantly shallow riffle habitat; mean and maximum depths within the site at low summer flow (August) were 20 and 50 cm, respectively and the substrate was about 60% cobble, 30% gravel, and 10% bedrock. At the times of sampling, water temperatures lay in the range 5° and 16°C, water conductivities 20-40 micro Siemens cm^{-1} (Table I).

Density estimates. The density of fish at the study site was estimated on nine dates: June, August and October, 1986; May, June, August and October, 1987; and May and June, 1988. On each date, barrier nets (0.3 cm mesh) were erected at the upstream and downstream boundaries of the site, and fish densities were estimated using electrofishing gear and the removal method (Zippin 1956; Seber 1982). For each estimate, 2 to 4 successive electrofishing runs were made. Capture probabilities were usually high (0.4 to 0.5), and the proportion of the total population (N) actually captured (T) during the survey usually exceeded 90%. Between each run, captured fish were counted, sampled (see below) and retained in stream-side cages; most fish were returned alive into the site following the final electrofishing run. Mortality resulting from electrofishing or sampling stress was usually less than 1% during the few hours that the fish were observed in cages.

Growth and production. Three age groups of *S. salar* and *S. trutta* were found: age 0 fry and ages 1 and 2 parr. Age 0 *S. salar* and *S. trutta* could be identified from length frequency data; ages of older fish were determined from subsamples. Subsampling included collecting scales from at least 3 fish of each modal length group and from all fish in intermediate size ranges. Most fish captured were measured for fork length (mm). For age 0 *S. salar* and *S. trutta* which were abundant, subsamples only were measured. Random subsamples of fish of all ages were also weighed in the field; weights of remaining fish were estimated from length-weight relationships established from the subsamples. Growth rates were estimated by comparing the mean weight of each year class on successive dates. Biomass (g m^{-2}) was calculated as the product of mean weight and population density (D m^{-2}).

Production rates (P) of *S. salar* and *S. trutta* were calculated as the product of instantaneous growth rate (G, % day^{-1}), mean biomass (B), and time (t, days), where $P = \Delta tGB$ (Ricker 1975). Average biomass (B) was calculated using formulae given by Ricker (1975).

Table I Estimated densities of fish (numbers m^{-2}) at the Mountain Brook site on nine sampling dates, 1986 to 1988. + indicates the species was captured but in low densities (<0.01 m^{-2}).

	1986			1987				1988	
	June 6	Aug 11	Oct 8	May 6	June 5	Aug 24	Oct 28	May 9	June 15
Temperature (°C)	12	16	6	8	11	12	9	5	12
Conductivity (µS cm^{-1})	30	-	20	20	25	40	25	-	30
Salmo salar	0.30	0.88	0.82	0.37	0.77	0.95	0.36	0.26	3.00
Salmo trutta	0.18	0.63	0.52	0.14	0.63	0.96	0.40	0.05	0.35
Salvelinus fontinalis	0.02	0.07	0.06	0.01	0.05	0.07	0.02	0.01	0.06
Petromyzon marinus	-	+	+	+	+	+	+	-	+
Gasterostidae	-	+	-	-	-	-	-	-	+
Anguilla rostrata	+	-	-	-	-	-	-	-	-
Total	0.50	1.58	1.40	0.52	1.45	1.98	0.78	0.32	3.41

Results

Density. The densities of *S. salar* ranged from 0.26 to 3.00 fish m^{-2}, and the densities of *S. trutta* from 0.05 and 0.956 fish m^{-2} (Table I). *Salvelinus fontinalis* were captured on each sampling date, but their density was low, < 0.10 fish m^{-2}. Ammocoete larvae (*Petromyzon marinus*), sticklebacks (Gasterostidae) and American eels (*Anguilla rostrata*) were also captured but their densities were low. Thus, salmonids dominated the fish community; total densities of the three species ranged bertween 0.32 and 3.41 fish m^{-2} (Table I).

S. salar and *S. trutta* were abundant enough to follow seasonal trends in density of individual year classes. Following a recruitment period between the June and August sampling period, most year classes decreased in abundance with respect to time (Fig 1). Judging from the size of fry (about 0.2g or less; Randall 1982), *Salmo* fry emerged from the gravel in early June each year. Because of their small size, newly emerged *Salmo* fry could not be identified for certainty as being either Atlantic salmon or brown trout. Relative abundance of the two species in June was calculated by applying the *S. salar/S. trutta* ratio observed in August to the total fry density estimated in June. In 1986 and 1987, both species showed a significant increase in densities of age 0 fish between June and August (Fig 1). This suggested that either emergence had not been completed by the June sampling dates, or that net immigration affected fry densities between these months. Following August, both year classess fluctuated somewhat between sampling dates, but generally decreased.

Growth. Length-weight regressions calculated the *S. salar, S. trutta* and *Salvelinus fontinalis* are compared in Table II. Regression coefficients (b=3) were not significantly different for any of the species. For estimating the mean weight of each year class on each sampling date, length frequencies were first converted to weight frequencies using these length-weight regressions.

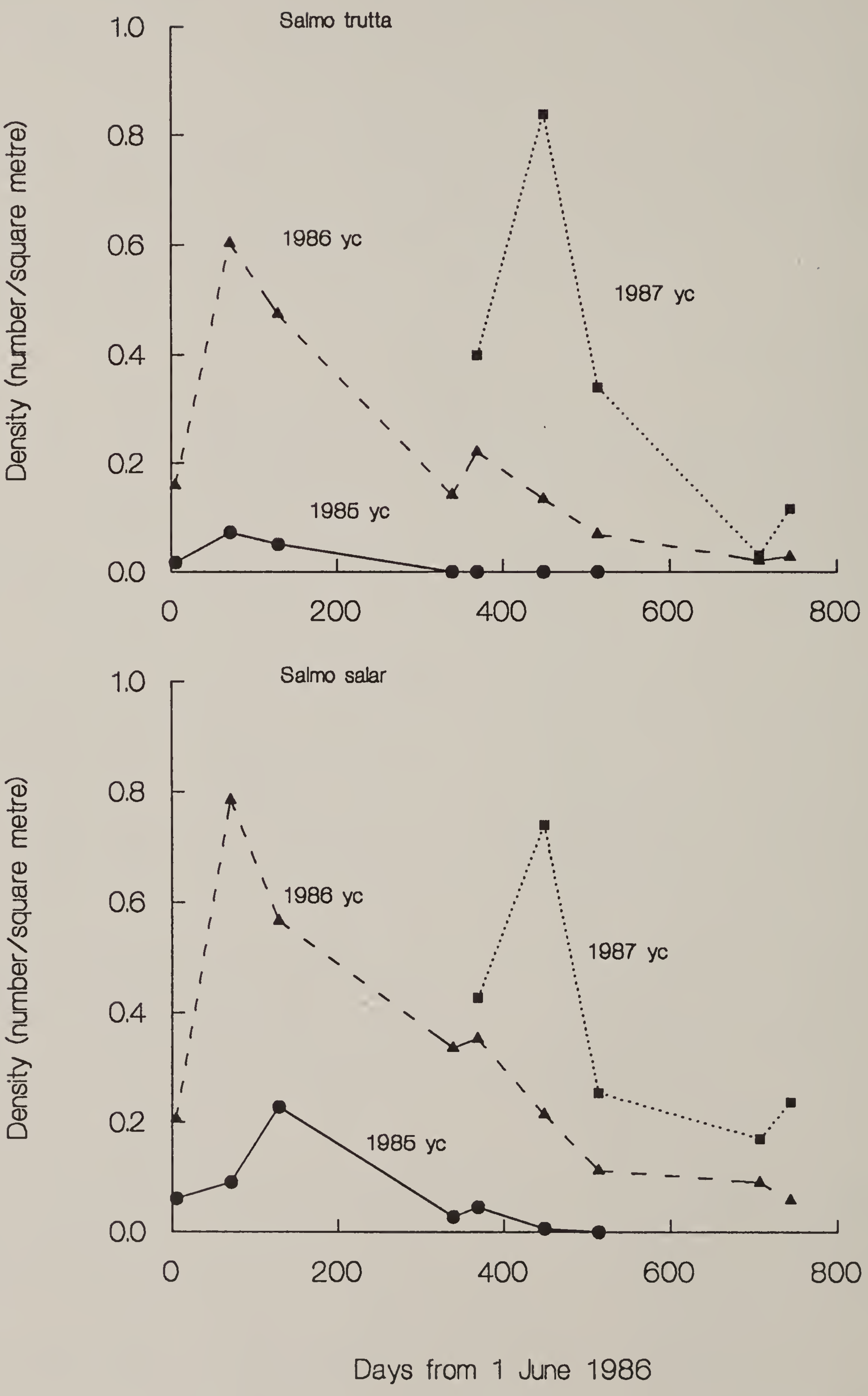

Fig 1 Density (numbers m^{-2}) of *S. salar* and *S. trutta* on nine sampling dates between June 1986 and June 1988 at Mountain Brook, Nova Scotia. Specific dates are given in Table I. Individual year classes (YC) are identified for each species.

Table II Regression equations for the relationship between wet weight (W, g) and fork length (FL, mm) of *Salmo salar, S. trutta,* and *Salvelinus fontinalis* at Mountain Brook. Regression equation: $\log_e(W) = \log_e a + b\log_e(FL)$.

Species	a (SE)	b (SE)	n	R²
Salmo salar	-11.45 (0.12)	3.03 (0.03)	407	0.97
Salmo trutta	-11.14 (0.11)	2.95 (0.03)	304	0.98
Salvelinus fontinalis	-11.40 (0.33)	3.01 (0.07)	26	0.99

Growth in length was restricted to a five to six month period each year. For *S. salar* and *S. trutta* of the 1986 year class, for example, mean size was similar on the October and following May sampling dates, indicating very little growth occurred during the winter months (Fig 2). Thus, although quantitative sampling was not feasible during winter because of snow and ice cover, apparently little growth was occurring during this time.

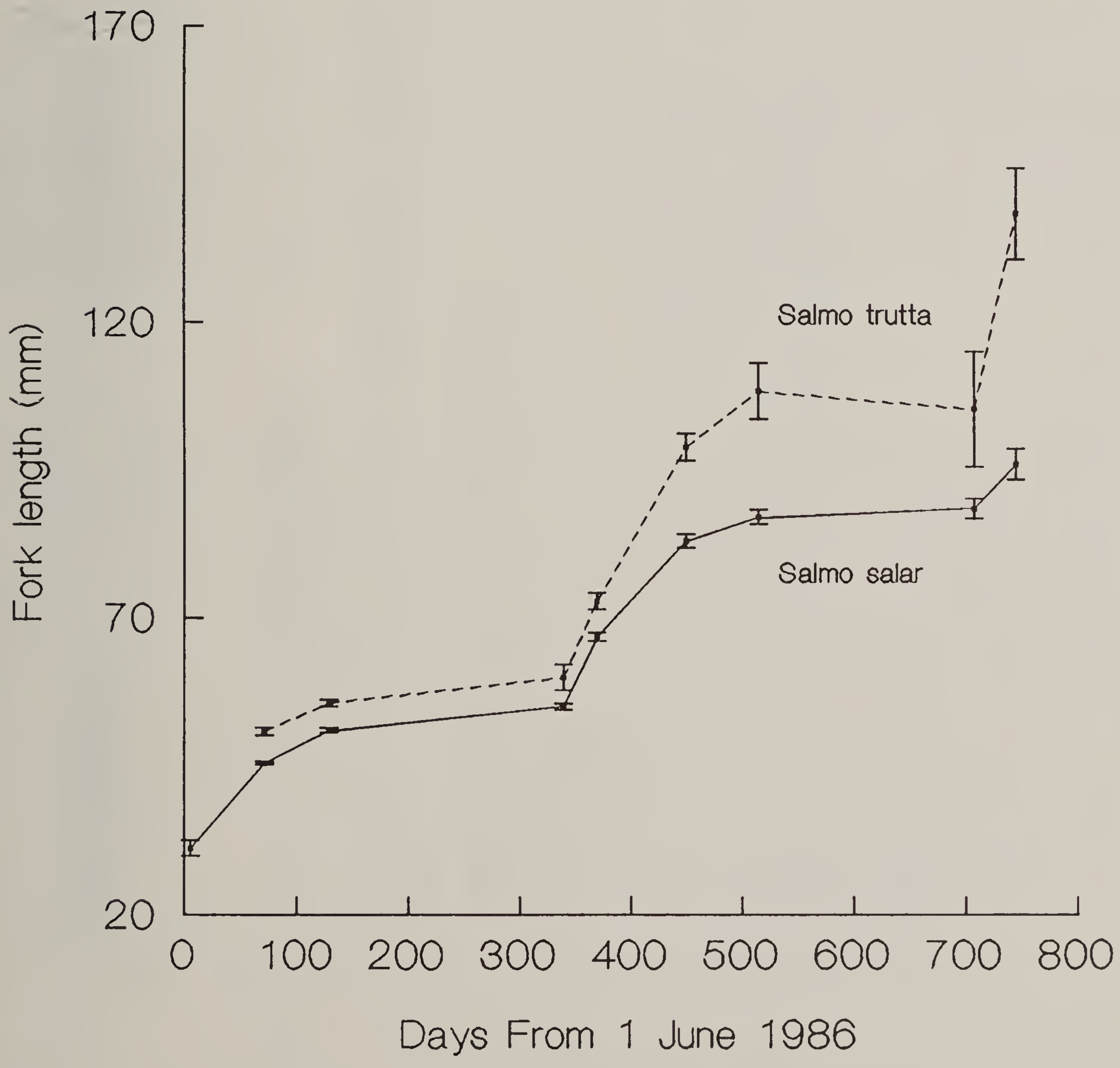

Fig 2 Seasonal growth of *S. trutta* and *S. trutta* at Mountain Brook, as indicated by increases in mean fork length with time of the 1986 year classes. Vertical lines indicate actual sampling dates and represent one standard error (SE).

Table III Comparison of mean length (FL, mm) of *Salmo salar* and *Salmo trutta* in August, 1986 and 1987. CV is coefficient of variation. Statistical differences in fork lengths between species were identified using *t*-tests.

		Salmo salar			*Salmo trutta*			
Date	Age	n	FL	CV	n	FL	CV	
August 1986	0+	141	45.4	8.2	108	50.7	13.4	(P<0.01)
August 1987	0+	128	45.7	6.4	148	49.1	11.6	(P<0.01)
August 1986	1+	14	84.6	7.6	13	109.0	12.5	(P<0.01)
August 1987	1+	40	83.2	8.7	26	99.1	11.8	(P<0.01)

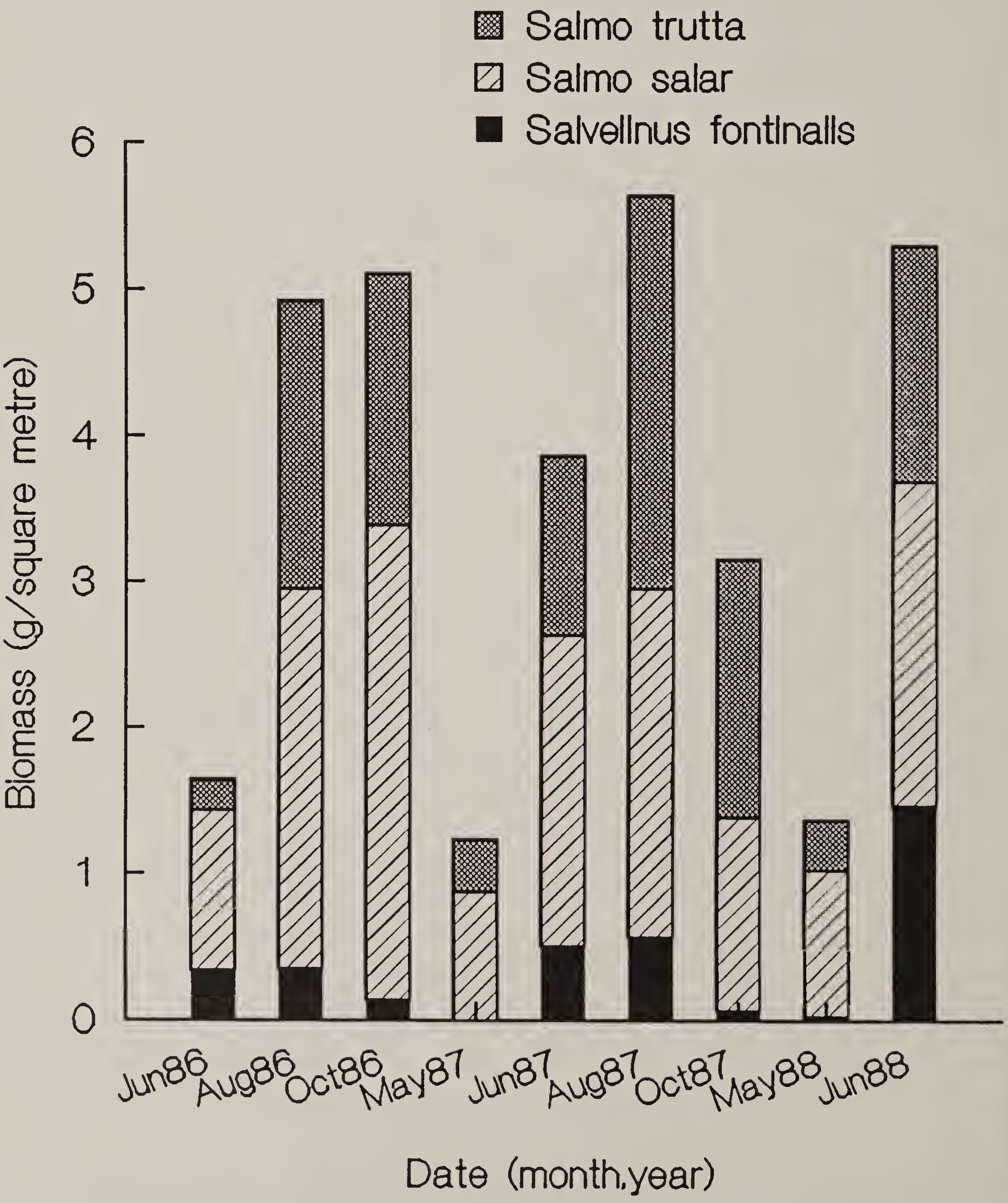

Fig 3 Biomass (m^{-2}) of salmonid fish at Mountain Brook on nine sampling dates.

Growth rate of *S. trutta* was greater than for *S. salar.* Mean size at age of *S. trutta* was consistently greater than for *S. salar,* and the relative difference increased during the growing season (compare the slopes of the growth curves for *S. salar* and *S. trutta* in Fig 2). *S. trutta* collected during August and October of 1986 and 1987 were significantly larger than *S. salar* (Fig 2 and Table III). Also, coefficients of variation (CV) were greater for each age group of *S. trutta* than *S. salar* (Table III). By the end of their second growing season (age 1 fish in October), the fork length of *S. trutta* lay in the range 85-142 mm and their weight 7.6-32.2 g. By contrast the analogous statistics for *S. salar* were 73-99 mm and 4.6-11.2 g.

Biomass and production. Biomass (g m^{-2}) of salmonids at the Mountain Brook site on each sampling date was estimated as the product of mean live weight and density. Total biomass ranged between 1.23 and 5.63 g m^{-2}, and was generally least in May and greatest in August and October (Fig 3) after the age 0 fish had fully recruited to the site and seasonal growth had occurred. The biomass of *S. salar* exceeded the biomass of *S. trutta;* average biomass for the nine sampling dates was 1.9 g m^{-2} for *S. salar* and 1.3 g m^{-2} for *S. trutta.* Biomass of *Salvelinus fontinalis* was low (average of 0.4 g m^{-2}; Fig 3).

The production of *S. salar* and *S. trutta* over the two years were similar; *S. trutta* contributed 42% of the total *Salmo* production the first year and 56% the second year (Table IV). Total annual production increased from 3.5 g m^{-2} y^{-1} for the period June 1986 to June 1987 to 5.9 g m^{-2} y^{-1} for the period June 1987 to June 1988 (Table IV). Increases in production between the two periods resulted from the greater density and biomass of the age 1 fish in the latter period (Table IV). Growth rates during the two periods were similar (Table III). Densities of *Salvelinus fontinalis* were too low to estimate the production of this species.

Discussion

The average total production rate of *Salmo* spp. at Mountain Brook in Nova Scotia over the two years was 4.7 g m^{-2} y^{-1}. As *S. salar* and *S. truttas* contributed 90% of the fish biomass (Fig 3), this rate was a rough estimate of the total community production rate at this site. Fish production at Mountain Brook was within the range observed for other rivers flowing into the Gulf of St. Lawrence (Table V), where the range of annual production was 0.3 to 6.3 g m^{-2} y^{-1}. Although production by the two sympatric salmonids at Mountain Brook was relatively high, it was not as high as allopatric brook

Table IV Comparison of the annual production (g $m^{-2}y^{-1}$) of *Salmo salar* and *S. trutta* at Mountain Brook, 1986 and 1987.

Period	Year class (age)	*Salmo salar*	*Salmo trutta*	Total	% *S. trutta*
June 86	1984 (2)	0.12	0.00	0.12	
to June 87	1985 (1)	0.42	0.18	0.60	
	1986 (0)	1.49	1.29	2.78	
	Total	2.03	1.47	3.50	42%
June 87	1985 (2)	0.04	0.00	0.04	
to June 88	1986 (1)	1.26	1.82	3.08	
	1987 (0)	1.31	1.47	2.78	
	Total	2.61	3.29	5.90	56%

Table V Comparative production rate of salmonids in rivers flowing into the Gulf of St. Lawrence.

River	Location	Predominant species	Production ($g\ m^{-2}\ y^{-1}$)	Reference
Philip	Nova Scotia	*Salmo salar* *Salmo trutta*	4.7 (3.5-5.9)	This study
Miramichi	New Brunswick	*Salmo salar*	2.5 (0.3-5.1)	Randall & Paim 1982
Matamek	Québec	*Salvelinus fontinalis*	3.5 (1.5-6.3)	O'Connor & Power 1976
Western Arm	Newfoundland	*Salmo salar*	2.2 (1.6-2.9)	Chadwick and Green 1985)
Highlands	Newfoundland	*Salmo salar* *Salvelinus fontinalis*	2.1 (0.8-5.0)	Gibson and Myers 1986

trout production at Kaikhosru Creek in Québec, where production over two years was estimated to be 6.1 and 6.6 $g\ m^{-2}\ y^{-1}$. Production at Kaikhosru Creek was high because of the good nursery habitat and the high biomass of age 0 trout in this stream (O'Connor & Power 1976). It may also be significant that fish populations in Québec were unexploited, while salmon and trout at all other locations cited in Table V were harvested. Production and yield of salmonids in eastern Canada is directly related to spawning stock, which in turn is influenced by fishing mortality (Chadwick 1985). Production rates of about 6 to 7 $g\ m^{-2}\ y^{-1}$ may represent the maximum for Gulf of St. Lawrence rivers. In European rivers the yield of salmonids is higher, sometimes exceeding 20 $g\ m^{-2}\ y^{-1}$, due to the greater productivity of rivers in Europe than rivers in eastern Canada (Gibson & Myers 1986).

The production of naturalized *S. trutta* at the Mountain Brook site was about equal to the production of native *S. salar* during the study period. Individual population traits that contributed to production however differed between the two species. *S. salar* were usually more abundant than *S. trutta* at the time of sampling, but their seasonal growth rates were significantly less. These differences between *S. salar* and *S. trutta* in population densities and growth rates counter-balanced one another to some extent and resulted in similar production rates.

Greater growth rates of *S. trutta* than *S. salar* have been observed often where the two species live sympatrically (Egglishaw & Shackley 1973; 1977; Gibson & Cunjak 1986Z). In a Scottish stream, where the population dynamics of the two species were compared for ten years, Egglishaw & Shackley (1977) found that the larger size of *S. trutta* resulted from a longer growing season than *S. salar*; *S. trutta* emerged earlier and continued to grow later in the fall than *S. salar*. Growth rate of *S. salar* during the spring/summer growth period actually equalled or exceeded the growth rate of *S. trutta* in this Scottish stream. In contrast, growth of *S. trutta* at Mountain Brook exceed that of *S. salar* during the summer months, indicating that mechanisms other than length of growing season caused the different growth of the two species. At Mountain Brook, sampling was not frequent enough to compare emergence dates of the *S. salar* and *S. trutta* fry, and therefore it was not possible to identify exactly when the *S. trutta* fry initially achieved their growth advantage over *S. salar*.

Given the characteristics of the stream habitat at the Mountain Brook site, densities of *S. trutta* relative to *S. salar* were surprisingly high. As noted earlier, the Mountain Brook site was predominantly riffle habitat, with water currents, depth and substrate characteristics ideally suited for juvenile *S. salar* (Symons & Heland 1978; Morantz et al. 1987). *S. trutta,* in contrast, prefer deeper and slower water (Egglishaw & Schakley 1982; Kennedy & Strange 1982; Gibson & Cunjak 1986). In their Newfoundland study, Gibson & Cunjak (1986) found that the two species were usually spatially segregated, with *S. trutta* older than age 0 preferring deeper, slower water than *S. salar,* although the differences were not statistically significant at all the sites studied. They also noted that overhanging cover can substitute for depth for *S. trutta.* Alders and other streamside vegetation provided abundant shade at Mountain Brook, and this may account for the relatively high *S. trutta* densities at this riffle site. Despite differences in preferred habitat, there is obviously considerable overlap in the type of habitat utilized by each species.

At River Philip, the above observations on the growth, relative density, and production rate indicated *S. trutta* have become established, and they are cohabiting successfully with native *S. salar* at this localized site. Obviously, interpretations of the relative status of the naturalized *S. trutta* popultion from this study are limited because of the small area surveyed. Information on harvests of *S. salar* and *S. trutta,* however, confirm that *S. trutta* populations have become well-established over extensive areas; estimates of angling catches from Cumberland county, where River Philip is a major stream, indicated that landings of *S. trutta* usually equalled or exceeded landings of *S. salar* in this region (Table VI).

Kennedy & Strange (1986) have found that juvenile *Salmo salar* living allopatrically survived twice as well and grew larger than when living sympatrically with *S. trutta.* Similarly, the latter species has replaced the native *Salvelinus fontinalis* population in some rivers in the United States (Fausch & White 1981; Waters 1983). Concern about the expansion of *S. trutta* into the native *Salmo salar* habitat in the Avalon peninsular, Newfoundland prompted a study of the interactions of the two species (Gibson & Cunjak 1986). These authors concluded that extension of the range of *S. trutta* in Newfoundland will probably be limited, but they warned that deliberate stockings to extend the range of the species should be discouraged until further research was done. The work reported in this paper shows that production of *S. trutta* and *S. salar* in a riffle habitat in a small Nova Scotia stream was similar; hence the concerns and conclusions of the Newfoundland workers apply equally to Nova Scotia.

Table VI Numbers of *Salmo salar* and *S. trutta* landed by anglers in Cumberland County, Nova Scotia, 1980-1987.

	Angling catch (numbers)	
Year	*Salmo salar*	*Salmo trutta*
1980	110	289
1981	99	292
1982	349	300
1983	964	442
1984	370	383
1985	204	536
1986	206	934
1987	N/A	252

Data sources - *S. salar:* Swetnam and O'Neil 1984; O'Neil et al 1985; 1986; and 1987. *S. trutta*: N. Adams, Nova Scotia Department of Lands and Forests, personal communication.

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PHYTOPLANKTON AND WATER QUALITY OF THE COASTAL WATERS OF NEW BRUNSWICK AND ADJACENT AREAS

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This paper gives the results of investigations during 1971 to 1984 on the phytoplankton of the coastal and marine waters of New Brunswick and Soctia-Fundy-Cody and Georges Bank regions. The phytoplankton composition, distribution and seasonal succession in relation to water quality are presented.

Le présent travail rapporte les résultats des études sur le phytoplancton des eux côtières et marines du Nouveau-Brunswick ainsi que des régions de Scotia-Fundy-Cody et du Banc Georges entre 1971 et 1984. La composition, distribution et succession saisonnière du phytoplancton en relation avec la qualitéde l'eau sont présentées.

Introduction

The coast of New Brunswick, Nova Scotia, and Prince Edward Island is long and its coastal waters are productive. Part of this productivity is related to the phytoplankton which sustains, in part, an important shellfish industry. Thus estiminations of the phytoplankton populations of these waters provide some understanding of this fishery. The purpose of this paper is to report the phytoplankton populations collected in the period during 1971-84.

Methods

Collection areas. In the study area several phytoplankton samples were collected during investigations on water quality, nutrients and biomass. The results on water quality have been reported (Gordon and Dadswell, 1984; Gordon et al., 1985; Jonnavithula, 1980; Lakshminarayana, 1976, 1983; Lakshminarayana and Bourque, 1979; Lakshminarayana and Jean-Pierre, 1975; Lakshminarayana and Jonnavithula, 1989). For convenience, based on the period and duration of investigation, the study area is divided into six regions. Five of these regions and the time and locations of collections are given in Table I. Data for the sixth region (Malpeque Bay, P.E.I.) have been published (Sita Devi and Lakshminarayana, 1989).

Methods of collection and identification. Phytoplankton samples were collected by both water bottles and 64 µm mesh net. The samples were preserved in Lugol's iodine-formalin neutralized with sodium tetraborate (pH 7.0-7.3). Methods of study and identification are given in Lakshminarayana (1983). Physico-chemical analyses were by the methods described by Sita Devi and Lakshminarayana, (1989).

Results and Discussion

Analyses of the coastal and marine waters are summarized in Table I. General distribution patterns of pH, temperature, salinity, nitrate and phosphate were variable but within the ranges reported (Tait and De Santo, 1975) for coastal and marine waters. These waters were well oxygenated. Higher concentrations of trace metals were noted

Table I Mean values of acidity, temperature and chamical constituents of marine waters off the coast of New Brunswick

Collection Areas	Year	pH	Temp. °C	Dissolved Oxygen $mg\ mL^{-1}$	Salinity 0/00	Nitrate $mg\ mL^{-1}$	Phosphate $mg\ mL^{-1}$	No. of analyses
CHALEUR 66°W 48°N to 65°W 47°N Jacquet R. to Lower Caraquet:								
1. June	1973	8.0	19.5	8.3	10.0	0.7	0.3	57
2. July	1973	7.9	21.5	8.0	14.6	0.9	0.15	57
BATHURST HARBOUR:								
3. July-September	1971	N.D.	16.6	6.7	23.6	N.D.	N.D.	12
4. June-November	1972	N.D.	15.5	8.6	10.7	N.D.	N.D.	9
Caraquet to Baie du Vin:								
5. June-November	1982	8.0	19.6	N.D.	19.9	N.D.	N.D.	72
Caraquet Bay:								
6. June-November	1982	8.0	13.6	8.1	27.4	N.D.	N.D.	48
NORTHUMBERLAND STRAIT 65°W 47°N to 62°W 46°N Shediac Bay:								
1. May	1972	7.6	9.9	9.0	11.0	N.D.	N.D.	180
Bouctouche Bay:								
2. June-August	1973	7.5	20.8	8.5	21.0	N.D.	N.D.	54
Northumberland Strait:								
3. June-August	1975	8.1	17.0	N.D.	23.7	0.03	0.02	95
4. May-October	1976	7.9	13.4	6.2	24.6	0.02	0.03	185
5. May-October	1977	8.1	11.6	6.9	25.7	0.06	0.01	185
GEORGES BANK[+] Shelf area of Nova Scotia & Georges Bank								
1. August	1974	N.D.	14.8	N.D.	32.8	0.7	0.3	47
BAY OF FUNDY[*]								
1. August	1978	N.D.	14.2	9.5	30.8	0.3	1.05	N.K.
Cumberland Basin:								
2. March	1979	N.D.	1.8	N.D.	29.6	12.7	1.2	N.K.
3. October	1979	N.D.	10.9	N.D.	33.6	5.3	1.1	N.K.
4. February	1980	N.D.	2.0	N.D.	30.5	12.7	1.2	N.K.
PASSAMOQUODDY BAY								
1. August	1977	8.0	N.D.	11.2	32.5	N.D.	N.D.	12
2. May	1978	8.1	4.7	12.3	26.8	N.D.	N.D.	14
3. February	1979	N.D.	1.3	N.D.	30.7	N.D.	N.D.	4
4. November	1979	8.2	9.3	3.5	32.5	0.01	0.9	18
5. November	1980	8.1	7.8	5.6	32.7	0.1	0.1	18
6. February	1981	8.0	6.8	8.5	31.9	N.D.	N.D.	18
7. November	1981	8.0	6.8	5.6	32.7	0.1	0.1	18
8. April	1983	8.1	1.5	12.0	33.3	0.1	1.1	18

[+] Subba Rao, 1975; [*] Keizer *et al.*, 1984; N.D. Not determined N.K. Not known.

Table II Bacillariophyceae collected in marine waters off the coast of New Brunswick and adjacent areas.

Genus†	Species	Chaleur	Northumberland Strait	Scotian Shelf & Georges Bank	Bay of Fundy	Passamoquoddy Bay
Achnanthes	*longipes*		4			2
	taeniata	5,6	4			1
Actinocyclus	*octanarius*	5,6	4		2,3	
Actinoptychus	*senarius*	5,6	4*		2,3*	4,5,7
Amphipleura	*rutilans*	1,2,3				
Amphiprora	*alata*	5,6	4			1,2
	ornata		4			
Amphora	*aungusta*		4			
	coffeaeformis	5,6				
	ovalis					1,2
Asterionella	*glacialis*	5,6	2,4,5	1	2,3,4	
Auliscus	*incertus*					3,4,5
Bacillaria	*paxillifer*				3,4	6
Bacteriastrum	*hyalinum*	1,2				
Bacteriosira	*fragilis*		2,5			2
Bellerochea	*malleus*	2				
Biddulphia	*alternans*		4*		2	8
	aurita	5,6	4*		2*	3,4,6,7
	mobiliensis				2,3*	3,4,6,7
	obtusa	2				
	pulchella		4	1		1
	regia					1
Brebissonia	*boeckii*					1,2
Caloneis	*thuretii*					1
	westii					4
Ceratulina	*pelagica*		4			1
Cerataulus	*turgidus*					1
Campylodiscus	*fastuosus*	2				
Chaetoceros	*affine*	5	4	1	2,3	
	atlanticum	5,6	4*	1*		2,1
	breve		2,4			
	boreale					3
	compressus	5,6*	4,5*	1	1	1,2
	concavicorne	1,2				8
	coronatum				2,3	
	constrictum		2,4			1,2*
	convolutum					3,8
	curvisetum					3,4,6,7
	danicum		2			3-8
	debile	5,6	2,4*			3-8*
	decipiens	5*	2-5*		1	1-7
	diadema	5	2		1,3,4	1,2*
	didymum	5	5		1	1
	furcellatum		2			1
	gracile				2,3	
	laciniosum	5,6	2,4			1
	lorenzianum	5,6	1,2			1

Table II (cont'd)

Genus[†]	Species	Chaleur	Northumberland Strait	Scotian Shelf & Georges Bank	Bay of Fundy	Passamoquoddy Bay
Chaetoceros	*peruvianum*				2	
	radians				2,3	8
	septentrionale	5	2,4,5			
	sociale	5,6	2,4*			1*
	subtile				2	
	teres	5,6	2,45			1
	wighami	5,6				
	willei	5,6	1,2			
Cocconeis	*disculoides*	5,6				
	scutellum	5,6*	4,5			
Corethron	*criophilum*					6,7,8
Coscinodiscus	*asteromphalus*		4*		2,3,4	6,7,8
	centralis	1,2,4*		1,4*	5,7*	
	concinnus	5	4*		2,4*	5,7*
	eccentricus		4*		2,3*	1,2
	lineatus				2,3	
	marginatus				2,3	2-7
	normani				2	3-7
	oculus-iridis				2	8
	radiatus	5,6*	1,2,4*	2,3,4	8	
Coscinosira	*polychorda*	6	4			
Cyclotella	*compta*		4			1
	meneghiniana				4	
	striata		4		2,3,4	
Detonula	*confervacea*		1,2			1,2
Diploneis	*crabro*	5,6				
	didyma				4	3-7
	sithii var. *smithii*				2,3	1
Ditylum	*brightwellii*				2,3	5,6,7
Endictya	*oceanica*					1,2,3
Epithemia	*musculus*					1,2,3
Eucampia	*radiatus*		2,4		2,3	
	zodiacus				2,3*	3-7
Eupodiscus	*radiatus*				2,3	1,2
Fragilaria	*capucina*		4			2
	crotonensis	5,6	5		2,3	
	oceanica	5,6	4		2,3*	
Gomphonema	*acuminatum*					1,2
	marinum					1,2
Grammatophora	*marina*	5,6	4,5			3-7
	oceanica		4			
	serpentina					3-7
Guinardia	*flaccida*					8
Gyrosigma	*balticum*	5	2,4,5			
	fasciola var. *fasciola*					2,3* 3
	hippocampus	5,6				
	wansbeckii				2,3	
Hyalodiscus	*scoticus*	5,6				3-7
Isthmia	*enervis*					1,2
	nervosa					1,2

Table II (cont'd)

Genus†	Species	Chaleur	Northumberland Strait	Scotian Shelf & Georges Bank	Bay of Fundy	Passamoquoddy Bay
Leptocylindrus	*danicus*	5,6		1*	3	1,2*
Lauderia	*borealis*	5,6				
Licmophora	*baileyi*		4,5			
	flabellata		4,5		4	
	gracilis				4	8
	lyngbyei		4,5			
	paradoxa	5,6				
Mastogloia	*elliptica*		4			
	smithii					1,2
Melosira	*artica*	5,6	4			
	crenulata					1,2
	juergensii				2,3	3-7
	moniliformis	1	4,5*		2,3,4	1,2*
	nummuloides		2,4		2,3,4*	3-7
	subflexilis					1,2
	sulcata		2,4		4	
	varians		4*			1,2
	westii				4	
Navicula	*abrupta*		4			1,2
	bahusiensis				2,3,4	3-7
	crucifera					3
	cryptocephala var. *Grunow*					3
	distans				2,3	1,2
	inclementis					1,2
	inflexa					1,2
	maculosa					1,2
	marina					1,2
	membranacea				2	
	perigrina				2,3	1
	scopulorum	5,6				
	vanhoffeni	5,6			2,3	
Navicula sp.		5,6				
Nitzschia sp.		5,6				
	acuminata		4			
	angularis		4		4	
	bilobata					1
	closterium	5,6*	1-5*		2,3,4	307
	delicatissima				2,3	
	dubia				2,3	
	frigida	5,6				
	lanceolata	5,6				
	longissima	5,6*	4,5*	1	2	
	paradoxa	5,6				
	panduriformis	5,6				
	plana	5,6				
	punctata	5,6				
	sigmoidea		45			
	seriata	5,6	2,4*	1*	2,3*	1-7
	sigma var. *intercedens*	5,6*	5			

Table II (cont'd)

Genus[†]	Species	Chaleur	Northumberland Strait	Scotian Shelf & Georges Bank	Bay of Fundy	Passamoquoddy Bay
Phaeodactylum	*tricornutum*		4			
Paralia	*sulcata*	1,2-6	4*		3,4*	3-7
Pinnularia	*ambigua*	5,6				
	cruciformis				2,3	
	major					1-7
	rectangulata	5,6				
	quadratarea				3*	
Planktoniella	*sol*					3-7
Pleurosigma	*aestuarii*		4*	1	2,3	
	angulatum					1,2
	attenuatum					1,2
	balticum					1,2
	decorum					8
	delicatulum					3-7
	elongatum	5,6				1,2
	fasciola		4			1,2
	formosum		1,2			1,2
	intermedium					3-7
	normani				2,3*	
	obscurum		4			
	spenceri					5,6,7
	strigosum				3	7
Porosira	*glacialis*		1,2			
Raphoneis	*amphiceros*					7
Rahbdonema	*arcuatum*					3-7
Rhizosolenia	*alata*	5,6	4		3*	1,2
	alata forma *gracillima*					1,2
	delicatula					8
	fragilissima		2,4			
	hebetata		4	1	2,3,4	3-7
	hebetata forma *semispina*			2		2,3,4
	imbricata		4			
	pungens	5,6*	2,5			
	robusta				4	
	setigera		2,4	1	2,3	1,2*
	stolterforthii				1	3
	shrubsolei					1,2
	styliformis	5,6	4	1	2,3*	3-7
Rhopalodia	*gibba*					1,2
Schroederella	*delicatula*			1		
Schroederella	sp.			1		
Skeletonema	*costatum*	5,6	2,4,5	1*	2,3	3-7*
	subsalum		4			
Stauroneis	*anceps*		4			
	salina		4			
Stauroneis sp.		4				
Stephanodiscus	*dubius*		4			
Stephanopyxis	*turris*		4			
Streptotheca	*tamesis*				2,3	
Striatella	*unipunctata*		4			1

Table II (cont'd)

Genus[†]	Species	Chaleur	Northumberland Strait	Scotian Shelf & Georges Bank	Bay of Fundy	Passamoquoddy Bay
Surirella	*fastuosa*		4		2,3,4*	
	gemma		4			1,2
	ovalis		4			3-7
	ovata				2,3*	
	smithii					3-7
Synedra	*affinis*	1,3,5,6*	4		2,3*	
	crystallina	4,5				
	fulgens	4,5				
	gallionii	4,5				
	investiens					3-7
	puchella				2,3	3-7
	radians					7
	ulna		4			
	undulata					3,7
Thalassionema	*nitzschoides*				1,2,3	1,3,4,6,7
Thalassiosira	*baltica*		4,5			
	decipiens		4*		2,3	
	gravida	5,6	2		2,3	1,2
	hyalina		4*			
	nordenskioldii	5,6	2,3,4		2	1,2
	subtilis		4			
Thalassiothrix	*frauenfeldii*			1	2,3	
	longissima	5,6	4		3,4*	7
	nitzschoides	5,6	4			1,2
Triceratium	*favus*					3-7
Triceratium sp.						3-7

[†] Names of genera are quoted only on first occurrence and are implied thereafter.

in the nearshore waters than the offshore waters (Lakshminarayana and Jonnavithula, 1989).

A total of 339 species representing 111 genera of phytoplankton were identified in this study, and are listed in Tables II and III. Fifty of these species were found in Malpeque Bay and not elsewhere, and 161 of the 339 species were not found in Malpeque Bay. The species reported were divided among Bacillariophyceae - 267, Dinophyceae - 50, Chrysophyceae - 5, Cryptophyceae - 2, Haptophyceae - 6, Euglenophyceae - 1, Prasinophyceae - 1, Cyanophyceae - 5, and Chlorophyceae - 2. Ultraplankton (< 2 µm) in the samples were generally abundant and this group of algae which were not identified, needs further study.

Phytoplankton of Chaleur (Fleuve du Jacquet to Baie du Vin). A total of 55 species were identified from 252 samples. The most commonly collected phytoplankton (given in Tables II and III) consisted primarily of diatoms. *Skeletonema costatum, Asterionella glacialis, Bacillaria paradoxa, Chaetoceros marina* and *Melosira moniliformis* were noted in October. *Peridinium ovatum, P. depressum, P. longipes* and *Dinopnysis* sp. occurred in small numbers in October and July and *P. depressum* was also observed in August. *Oscillatoria* sp. was seen in July in a few samples.

Table III Chrysophyceae, Dinophyceae, Haptophyceae and other phytoplankton collected off the coast of New Brunswick and adjacent areas.

Genus†	Species	Chaleur	Northumberland Strait	Scotian Shelf & Georges Bank	Bay of Fundy	Passamaquoddy Bay
Distephanus	*speculum*		4,5			8
	speculum var. setenarius					8
Dinobryon	*balticum*					3-7
Amphidinium						3-7
Ceratium	*arcticum*	5,6	1,2			
	bucephalum	5,6				
	extensum				2,3	
	furca				2,3	3-7*
	fusus	5,6	2,4	1	2,3	3-7*
	hirundinelle	1,2				
	lineatum		4		2,3	
	longipes	5,6	2,4*	1	3	
	macroceros	4*			2,3	
	symmetricum	5,6				
	tripos		4		3*	3,5*
Dinophysis	*acuminata*	5,6	4*			
	catenata					3-7
	caudata			1		
	ellipsoides		2			
	norvegica	5,6	2,4	1		1,2
	ovum			1		
	rotundata	5,6				
Diplosalis	*lenticula*	5,6				
Goniodoma	*depressum*	5,6				
Alexandrium	*tamarense*					1,2*
Gonyaulax	*spinifera*		4			
Protogonyaulax	*tamarensis*		4	1	2,3*	
Gymnodinium	*lohmanii*	5,6				
Gymnodinium sp.				1		
Oxytoxum sp.			4	1		
Peridinium	*achromaticum*	5,6				
	brevipes	1,2				
	crassipes	5,6	4*			
	depressum		2,4	1	1	1,2*
	longipes	5,6				
	ovatum	5,6				
	pallidum	5,6	1,2			
Phodolampas	*spinifera*	5,6				
Prorocentrum	*micans*	5,6	4	1		
Phalacroma sp.			4	1		
Halosphaera	*viridis*	5,6				
Anacystis sp.	5,6					

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† Names of genera are quoted only on first occurrence and are implied thereafter.

Phytoplankton of the Northumberland Strait. The 100 species of phytoplankton noted in 699 samples from the Northumberland Strait were distributed among 68 genera and as six classes: Bacillariophyceae - 46; Dinophyceae - 11; Cryptophyceae - 2; Hepatophyceae - 6; Chrysophyceae - 2; and Euglenophyceae - 1. Frequent species found in this area are given in Tables II and III. During the spring, cold water species e.g. *Coscinodiscus asteromphalus, C. centralis, C. eccentricus, C. radiatus, Pleurosigma aestuarii, Thalassionema nitzischioides, Dinophysis acuminata, Chilomonas marina* were abundant. From May to June and briefly in September and October diatoms were dominant. The following species were only observed in May to October 1976: *Dictyocha fibula, Amphidinium longum, Dinophysis acuta, D. borealis, Exuviaella baltica, Glenodinium danicum, Gymnodinium gracile, Peridinium conicum, P. excentricum, P. pellucidum, Pyrocystis lunula, Prorocentrum rostratum, Protoceratium reticulatum, Chilomonas marina, Rhodomonas* sp., *Acanthoica acanthos, Phaeocystis sp., Pontosphaera huxleyi, Syracosphaera* sp., *Cocolithus pelagicus*, and *Euglena* sp. After August dinoflagellates were important.

Phytoplankton of the Scotian Shelf and Georges Bank In this region during August 1974 (Subba Rao, 1975) 109 samples from several sampling stations showed many microflagellates and several species of dinoflagellates, although the latter were not in large numbers. The diatoms *Skeletonema costatum, Leptocylindrus danicus, Nitzschia seriata, Chaetoceros atlanticum* and *Chaetoceros* sp. were frequent in the samples.

Phytoplankton of the Bay of Fundy. Examination of 1077 water samples collected on four cruises throughout the Bay at Pecks Cove and Cumberland Basin (Gordon and Dadswell, 1984) revealed 40 genera consisting of 119 species representing diatoms (34 genera or 105 species), dinoflagellates (4 genera or 12 species), silicoflagellate - 1 and green algae - 1. The benthic diatom *Paralia sulcata* was very common as was *Actinoptychus senarius*, an oceanic diatom. There appears to be a definite seasonal distribution of phytoplankton typical of a temperate coastal region having winter, spring, summer and autumn populations. Several species described by Marshall (1978), as occurring in the Gulf of Maine and surrounding Atlantic waters were represented among the species recorded from the Bay of Fundy (Tables II and III). The areas Chignecto Bay, Cumberland Basin, Minas Basin were rich or diverse in diatoms, the greatest diversity appearing in mid June. The dominant species found in Cumberland Basin are given in Tables II and III. These species were found in Chignecto Bay where *Coscinodiscus centralis, Cyclotella meneghiniana, Eucampia zodiacus, Thalassiosira decipiens* and *Ceratium tripos* were also common. Similar species were found in the Minas Basin, hence differences between the inner and outer Bay of Fundy were apparent.

Phytoplankton of Passamoquoddy Bay. The phytoplankton of the Quoddy region resembles that of the coastal margin of the Gulf of Maine. A checklist of 127 species of phytoplankton consisting of 120 diatoms, six dinoflagellates and one Chrysophyte has been published (Lakshminarayana, 1983). Species of *Chaetoceros* lead both in diversity and numbers in the Passamoquoddy Bay waters, and the diatoms increased with the onset of spring. *Thalassiosira nordenskioldii, Rhizosolenia setigera, R. styliformis* were most frequent in March and April. *Thalassiosira gravida, Chaetoceros debile, Ch. diadema, Ch. constrictum, Ch. sociale, Coscinodiscus concinnus* and *C. centralis* were common in summer while at the end of summer *Skeletonema costatum, Leptocylindrus danicus* and *Rhizosolenia setigera* appeared in these waters. In general, the dinophycean members were few and they infrequently occurred in autumn and summer. These species were *Ceratium tripos, C. fusus, C. furca,* *Peridinium depressum, Dinophysis norvegica* and *Alexandrium tamarense (Gonyaulax excavata)*. A reported herring kill in the Bay of Fundy was attributed to the toxins of *A. tamarense* (White, 1977) during blooms of this species in the Atlantic coastal waters of northeastern America. During the winter *Skeletonema costatum* and *Melosira moniliformis* were common.

Water quality and phytoplankton distribution To understand phytoplankton distribution it is necessary to consider water quality characteristics as well as geographic, hydrographic and topographic parameters. Culture studies (Guillard and Kilham, 1977) on *Phaeocystis* and *Skeletonema* from tropical and northern coastal waters of the Atlantic Ocean show that the optimum temperatures of growth of these species depends on where they were collected. By contrast some authors (Braarud, 1945; Smayda, 1958) consider that the latitudinal distribution of phytoplankton (arctic, boreal, temperate, tropical) is regulated by temperature.

In the case of *Thalassiosira nordenskioldii* it was found that this species grew well at 10-11°C in culture, and our observations (Sita Devi and Lakshminarayana, 1989) showed that it was a dominant species in Malpeque Bay when the water temperature was 18-21°C. In the surveys reported in this paper (Table II) it was also found during the summer months when the water temperature was in the range 13-21°C. By contrast it has been reported (Gran and Braarud, 1935; Smayda, 1957) to achieve its maximum population density in the temperature range 2-3°C. This apparent anomaly may be due to the presence of high nutrient levels for Braarud (1945, 1945a) found that boreal diatoms grew actively at 20°C in polluted waters, whereas growth was negligible in non-polluted waters. Though our studies (Sita Devi and Lakshminarayana, 1989) indicated that the waters of Malpeque Bay were of good quality, there may still have been sufficient nutrient in spring and summer to account for the bloom of this organism. Hence, at the moment there are insufficient reasons to reclassify this organism from an arctic boreal neritic species (Davidson, 1931).

The optimum conditions of temperature and salinity for growth and reproduction of dinoflagellates and cocolithophorids remain obscure, though Raymont (1963) has reported that some species reproduce most actively at low salinities. Smayda (1958) quoted high temperature optima (20-25°C) for *Prorocentrum micans, Ceratium fusus* and *C. furca*, and our studies (Table III), in agreement, indicated that maximum populations of the first two organisms occurred during the summer (temperature range 13-21°C). However, *C. furca* was isolated only during the winter months when water temperatures were in the range 1-11°C. *Dinophysis acuminata* was also found predominantly (Table III) in the summer months at ~14°C and salinity ~25‰ and these results agree with those of Lassus et al. (1985) who found that though blooms of this organism occurred between 30-35‰ salinity at 15-21°C, its preferred habitat lay in colder waters.

In summary the most common phytoplankter of the marine waters surveyed in this work were *Thalassiosira gravida, T. nordenskioldii, T. decipiens, Skeletonema costatum, Chaetoceros dicipiens, C. boreale, C. atlanticum, C. diadema, Rhizosolenia setigera, R. styliformis, Thalassionema nitzschoides, Thalassiothrix longissima, Distephanus speculum, Peridinium ovatum, Ceratium articum, C. fusus, C. longipes, C. tripos,* and *C. macroceros.* These species were found in a wide range of water temperatures and salinities.

Bacon (1977) observed that the spring phytoplankton of the Northumberland Strait probably forms an important food source for oyster (*Ostrea edulis*) populations. Of the 128 phytoplankton species recorded in British coastal waters, 33 were found in the stomach contents of marine animals (Hendey, 1964) and hence were part of their food supply. Thus certain species might well be useful as nutrients in pisciculture (Maestrini et al., 1984) or as assay organisms to measure specific pollutants. Phytoplankton identification will also help the selection and culture of specific algal ecotypes.

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TRACE ELEMENTS IN WATER, SEDIMENT, AND BIOTA OF THREE OLIGOTROPHIC LAKES IN QUEENS AND ANNAPOLIS COUNTIES, NOVA SCOTIA

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Concentrations of a range of elements have been measured in water, sediment, plankton, benthos, macrophytes and fish of three oligotrophic lakes in Kejimkujik National Park, Nova Scotia. Lead appeared to be concentrated in the top centimeter of undisturbed sediment cores; probably due to anthropomorphic use of leaded gasoline. The low concentrations of calcium and magnesium in these waters are probably due to the substratum of impoverished basement rocks and glacial moraine. High concentrations of sodium and chloride reflect the influence of atmospheric aerosols of marine origin.

Les concentrations d'une gamme d'éléments ont été mesurés dans l'eau, dans le sédiment, dans le plancton, dans le benthos, dans les macrophytes, et dans les poissons de trois lacs oligotrophiques dans le Parc National Kejimkujik en Nouvelle-Ecosse. Le plomb s'est avéré le plus concentré dans le cm. superficiel de noyaux de sédiment non-dérangés; ceci démontre une influence anthropogénique probablement reliée à l'usage d'essence au plomb. Les concentrations relativement basses du calcium et du magnesium dans ces eaux (comparées à des lacs oligotrophiques situés ailleurs) sont probablement une conséquence d'un fond de roche appauvri et de moraines glaciales. Les concentrations élevées du sodium et de la chlorure réfléchissent une influence d'aérosols atmosphériques provenant de la mer.

Introduction

Metals and other trace elements are emitted from various anthropogenic sources such as power plants, smelters and automobiles, and these contaminants are known to be transported in the atmosphere over long distances, after which they may deposit on forests or waterbodies (Freedman, 1989). In order to determine whether such atmospheric depositions cause measurable increases in the concentrations of potentially toxic trace elements in ecosystems, information is required on their background concentrations in the environment. To provide such background information, multielemental biogeochemical surveys have been made of putatively uncontaminated environments. Reviews of such data may be found in Bowen (1979) and Forstner and Wittmann (1981). Here we summarize the results of a study of the concentrations of

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26 elements in water, sediment, and biological compartments of three oligotrophic lakes in southwestern Nova Scotia, though not all elements were found in every compartment. The purposes of this study were to provide baseline data against which future changes in elemental concentrations might be assessed and to determine whether any of the elements were present in unusually large or small levels in these waterbodies.

Methods

Study area

The three lakes examined were Beaverskin, Pebbleloggitch and Kejimkujik, all located in Kejimkujik National Park in southwestern Nova Scotia. The physical, chemical and biological characteristics of the lakes and their watersheds have been described in detail (Kerekes and Freedman, 1989). Beaverskin and Pebbleloggitch Lakes are small, shallow, oligotrophic lakes in small headwater watersheds (Table I). Pebbleloggitch Lake has relatively dark and acidic water (87 Hazen units, pH 4.3), while Beaverskin Lake is clear and less acidic (5 Hazen units, pH 5.3). Kejimkujik Lake is larger and deeper, drains a large watershed and its colour and acidity (65 Hazen units, pH 4.9) is between the other two smaller lakes (Table I). The watersheds of the lakes are largely forested, but about 33% of the Pebbleloggitch watershed is a poor-fen dominated by heaths and *Sphagnum*. Parts of the Kejimkujik watershed are similar wetlands. It is because of the brownwater drainage from these wetlands that these two lakes are dystrophic.

Table I Location and selected morphometrics of the three study lakes.

	Beaverskin	Pebbleloggitch	Kejimkujik
location (grid reference)	140 085	124 078	
latitude	44°18' N	44°18' N	44°23' N
longitude	65°20' W	65°21' W	65°15' W
elevation (m a.s.l.)	120	120	90
lake area (km²)	0.443	0.338	24.35
drainage basin & lake (km²)	1.0	1.6	723
maximum depth (m)	6.8	2.5	19.2
mean depth (m)	2.8	1.6	4.4
water retention time (y)	1.36	0.38	0.17

Collection and preparation of samples Field sampling was done in mid-July, 1980, except for undisturbed sediment cores, which were collected in July 1986.

Water samples The lakes were sampled at their outflow. An acid-washed nalgene bottle of capacity 1 L was rinsed several times with lake water, filled, and the sample was preserved by adding nitric acid to pH ~2.

Sediment samples Duplicate samples of sediment were collected from a mid-lake deep water station, using an Ekman dredge. The sediment was taken from the center of the sampler, passed through a 0.5 mm nylon mesh sieve, and dried at 100°C. Subsamples of 5 g dry weight (d.w.) were digested in nitric-perchloric acids (5:2) to prepare them for chemical analysis. In addition, undisturbed sediment cores were collected from two of the lakes (Beaverskin and Pebbleloggitch) in 1986, by using divers to carefully insert and extract a 7.5 cm diameter, acid-washed, plexiglass tube at deepwater stations of the lakes. The sediment from these cores was sectioned into 1 cm depth segments, dried at 100°C, sieved through 1 mm sieves and subsamples (5 g, d.w.) digested in a teflon vessel in nitric-hydrofluoric-perchloric acids (3:3:1) at 100°C, prior to chemical analysis.

Plankton Duplicate plankton tows were made, using a 0.15 mm nylon mesh net. Samples were dried at 100°C, and subsamples (0.5 g d.w.) were digested in nitric-perchloric acids (5:2) to prepare for chemical analysis.

Benthos Benthic invertebrates were sorted from the sediment samples. Composite samples of various taxa (mostly *Asellus*, Diptera, *Hexagenia*, Odonata and Trichoptera) were dried at 100°C and subsamples (0.5 g d.w.) digested in the same way as the plankton samples.

Macrophytes Samples of *Eriocaulon septangulare*, *Nuphar variegatum* and *Utricularia vulgaris* were collected, rinsed to remove sediment, freeze-dried, homogenized in a powder mill and subsamples (0.5 g) digested as described.

Fish Individuals of brown bullhead (*Ictalurus nebulosus*), golden shiner (*Notemigonus crysoleucas*) and yellow perch (*Perca flavescens*) were collected by gillnetting. Composite samples of 3-5 individuals per species were homogenized in a blender, dried at 100°C, and subsamples (0.5 g d.w.) digested in nitric-perchloric acids (5:2) for chemical analysis. Samples for mercury analysis were kept frozen and were then digested and analysed.

Chemical analyses All chemical analyses of the samples collected in 1980 were made by Barringer Magenta Limited, Rexdale, Ontario. Analyses were done on a total of 17 samples of water, 16 of sediment, 94 of fish, 8 of benthic invertebrates, 15 of plankton, and 46 of macrophytes. Most determinations were by inductively coupled plasma emission spectroscopy using standard procedures. Cadmium, mercury and lead were analyzed by atomic absorption spectroscopy. Chromium in sediments was also analyzed by atomic absorption. Determinations were made of blank digests and all analytical data were corrected for these blank values. The ratio of sample:blank analyses was 8:1 for water and sediment, and 1:17 for biological samples. Standard reference materials: a syenite rock sample (S4-3) from Canada Center for Mineral and Energy Technology; a bovine liver standard and an orchard leaf standard from U.S. Bureau of Standards were analyzed as controls and to indicate analytical accuracy. The results are given in Table II.

The sediment core samples collected in 1986 were analyzed in the laboratories of OceanChem Ltd. Lead was determined by atomic absorption spectroscopy and the remaining elements by inductively coupled plasma atomic emission spectroscopy.

Data analysis Descriptive statistics were computed for each of the elements determined for the various samples from each lake (Ag was not detected). In cases where the concentration of an element was below the detection limit, the values were treated as 'missing' for the statistical analysis and are indicated in Tables III to VII with a blank.

Results and Discussion

The analyses of standard reference materials given in Table II indicate that the results obtained were generally within experimental error of the certified values; large percentage differences were generally found only for those elements present in very small quantities (<5 mg kg^{-1}). The exceptions to this rule were analyses of orchard

Table II Replicate (7) analyses of elements (mg kg^{-1}) in standard reference materials and comparison with their certified values (σ = standard deviation; n.a. = not available; % difference between certified and analytical values given in parentheses when > 5%)

Element	Orchard leaves			Bovine liver			Syenite rock		
	mean	σ	certified value	mean	σ	certified value	mean	σ	certified value
Al	174	11	n.a.	2.9	.3	2	59300	540	62400
Be				<.01		.017	18.4	1	20(8)
Ca	18600	460	20900(11)	125	3	124	56700	590	59000
Cd				<.25	.27				
Co	<.5		.2	1.3	.3	.21(!)	90	1	82(9)
Cr	1.4	.1	2.6(46)	.6	.2	.6	3.3	1.2	11(70)
Cu	10.3	.2	12(14)	178	3	193(8)	27	7	17(37)
Fe	240	5	300(20)	270	3	268	44700	500	44900
Hg				15	1	16			
K	13600	520	14700(7)	9870	200	9960	34640	240	34900
Mg	5800	100	6200	630	12	604	16150	170	16100
Mn	81	2	91(10)	10	.1	10.3	2320	20	2480
Mo				3.0	0	3.5(14)			
Na	63	7	82(23)	2470	30	2430	30830	220	30800
Ni	2.3	.1	1.3(77)	.7	.2	n.a.	7.3	.7	11(34)
P	1880	20	2100(10)	11600	200	11100	2410	30	2360
Pb	38	2	45(16)	<.5		.34	150	2	133(10)
Sr	25	1	37(34)	.07	.01	.14(50)	250	2	300(17)
Th	<.6		.064	.033	.016	.05	1240	3	1003(19)
Ti	4.9	.5	n.a.				970	15	900(7)
V				.13	.04	.1	56	1	50(11)
Zn	20	1	25(20)	136	3	130	233	5	244
Zr							520	8	320(38)

leaves where lower values for Ca, Fe, Mn, Na, P, Pb, Sr and Zn than the certified concentrations, were obtained. An explanation for these discrepancies is unknown but requires further investigation and may mean that the values given in this paper for plant material should be revised upwards. Standard analyses for Ba, Si and Sn were not obtained.

The analytical values for 20 elements in water samples from the three lakes are given in Table III; values for Cd, Co, Mo, Sn, Th, and Zr were below the detection limits. These analyses, when compared with those of benthic invertebrates (Table IV, Si not detected), plankton (Table V, Ba, Be, Pb & Si not detected), macrophytes (Table VI, Ba, Be & Si not detected) and fish (Table VII, Ba, Be & Si not detected), show that there was bioaccumulation of elements in all these compartments. However the biota did not have consistently greater or smaller elemental concentration than the sediments (Table IV).

Other than the general biomagnification of elemental concentrations compared with water, there was little food chain accumulation of the elements. In relatively uncontaminated environments like the lakes examined in this study, the relative concentrations among the various biological compartments depend more on element specific physiological requirements or sequestering mechanisms than on trophic level. For example cyanoglobin, is a copper containing respiratory protein found e.g. in molluscs, which species therefore, have a relatively high content of this element (Bryan, 1968; Hutchinson et al., 1976).

Table III Concentrations ($\mu g\ kg^{-1}$) of elements in the water of Kejimkujik, Pebbleloggitch and Beaverskin Lakes (σ = standard deviation; n = number of analyses)

Element	Kejimkujik			Pebbleloggitch		Beaverskin	
	Mean	σ	n	Mean	n	Mean	n
Al	100	0	7	190	3	50	3
Ba	2.4	.8	7	2	2	2.5	2
Be	.1		1				
Ca	670	80	9	340	4	350	4
Cr				1	1	.9	1
Cu	11		1				
Fe	200	60	9	105	2		
Hg	.1	.04	5	.09	3	.07	3
K	100	0	5	150	2	100	2
Mg	480	40	9	390	4	410	4
Mn	37	2	9	8	2	16	4
Na	3000	50	9	3000	4	2900	4
Ni	23	8	8				
P	9	1	9	14	4	5	4
Pb	2.2	.1	9	2.3	4	2.3	4
Si	473	160	9	600	4	150	3
Sr	5.2	1.6	8	4.5	3	3.7	2
Ti	1		1				
V						6	1
Zn	8	1	8	4	2	4.5	2

The trace element concentrations reported are similar to those of uncontaminated fresh waters [c.f. data in reviews of Bowen (1979); Forstner and Wittman (1981)], and as might be expected, differences between specific compartments were small, though some (see e.g. Table VII) were statistically significant. There were, however, several cases where the data diverged from that recorded for other pristine fresh waters and these are discussed below in greater detail.

Lead in sediments Uncontaminated soil typically contains about 35 mg kg^{-1} of lead (Bowen, 1979). The mean lead concentration found in the bulk sediments collected from the three lakes lay in the range 18 - 47 mg kg^{-1}, with a maximum of 65 mg kg^{-1} (Table IV). However values of 123 mg kg^{-1} were obtained on samples taken from the top centimeter of the sediment and the mean value for this horizon in Beaverskin Lake was 96 mg kg^{-1}. There was a pronounced vertical stratification in the sediments of Beaverskin and Pebbleloggitch Lakes (Table IV) where values >40 mg kg^{-1} were found in the upper 5 cm of the sediment horizons and <10 mg kg^{-1} at depths in the range 9-23 cm. Similar stratification was also found in nearby Big Dam Lake. This pattern of elevated concentrations of Pb in the surface sediments of remote lakes is probably caused by increases in the rate of atmospheric deposition of this element as a result of the long-range transport of emissions associated with automobile exhaust (Freedman, 1989). Enhanced deposition of lead to remote ecosystems has previously been attributed to automobile emissions, e.g. deposition to forested watersheds at relatively high elevation in the N.E. United States (Siccama and Smith, 1978; Andresen et al., 1980; Friedland and Johnson, 1985).

Elements of groups I and II The cations: Ca^{+2}, K^{+} and Mg^{+2} are present in relatively small concentrations in the water and sediments of the three lakes. Most notably, Ca^{+2} in water is present in the range .34 - .67 mg kg^{-1} (Table III), compared with Bowen's (1979) 'typical' value of 15 mg kg^{-1} for fresh water, or compared with continental soft

Table IV Concentrations of elements in the sediments and benthos of Kejimkujik, Pebbleloggitch and Beaverskin Lakes (σ = standard deviation; n = number of analyses)

Element	Kejimkujik					Pebbleloggitch				Beaverskin		
	Sediment			Benthos		Sediment		Benthos		Sediment		Benthos
						bulk	top 5 cm			bulk	top 5 cm	
	Mean	σ	n	Mean	n	Mean (n=4)	Mean (n=3)	Mean	n	Mean (n=4)	Mean (n=3)	n=1
(mg g^{-1})												
Al	85.6	7.4	8	.86	3	15.4	10.8	.1	2	27.5	18.0	.12
Ca	2.9	.19	8	.35	3	2.5	1.02	.17	2	2.2	1.37	.39
Fe	40.8	2	8	1.5	3	2.8	2.97	.13	2	6.8	5.5	.31
K	27.3	1.9	8	1.8	3	4.7		.75	2	8.8		2.08
Mg	9.7	.9	8	.43	3	.92	.49	.16	2	1.03	.77	.24
Mn	1.13	.13	8	.031	3	.057	.035	.002	2	.21	.243	.01
Na	11.8	.5	8	1.39	3	4.3		.68	2	6.7		1.78
P	.9	.06	8	1.88	3	.67	.73	.98	2	1.2	1.38	1.69
Ti	4.4	.5	8	.03	3	.73	.69	.007	2	.73	.63	.004
Zn	.12	.005	8	.04	3	.014	.006	.022	2	.051	.006	.027
(µg 10g^{-1})												
Ba							510				810	
Be						5	<10			8	13	
Cd							<10				<10	
Co	160	120	7	29	3		18	19	2		29	30
Cr	810	120	8	15	3	100	87	7	2	88	130	7
Cu	230	60	8	47	3	60	210	28	2	430	150	61
Hg	.22	.13	8	.42	1	.26		.23	1	.21		
Ni	4	4	6	13	3	43	49	18	2	50	71	13
Pb	180	40	8	25	1	340	430	20	2	470	560	
Sn							29				70	
Sr	1200	90	8	16	3	320	150	5	2	370	180	14
Th				17	3			10	1			30
V	880	120	8	18	3	97	150	5	2	160	220	
Zr	1040	50	8	10	1	200	220					

water lakes e.g. the Experimental Lakes Area (ELA) of N.W. Ontario (1.6 mg kg^{-1}; Armstrong and Schindler, 1971). This observation is related to the small quantities of calcium in the thin mantle of soil and till overlying the hard oligotrophic bedrock in this part of Nova Scotia (Freedman and Morash, 1985; Kerekes and Freedman, 1989). Because of the sparse quantities of these cations in the study lakes, their waters have little acid neutralizing capacity and are therefore susceptible to acidification caused by deposition of acidifying substances from the atmosphere and by input of organic acids from the sphagnaceous wetlands of their watersheds (Clymo, 1984; Gorham et al., 1984; Kerekes and Freedman, 1989).

The concentrations of Na^+ in the study lakes (range 2.9 - 3.0 mg kg^{-1}; Table III) are large compared with about .9 mg kg^{-1} for softwater lakes of the ELA (Armstrong and Schindler, 1971). This observation possibly reflects the facts that the lakes are less than 60 km from the Atlantic Ocean, so there is a relatively large deposition of marine derived aerosols (Freedman and Clair, 1987; Kerekes and Freedman, 1989).

Elements mobilized by acidity Aluminium is abundant as an insoluble constituent of various minerals of watershed soils and aquatic sediments (in which its 'typical total' concentration is *ca.* 7%) and it is known that acidic water can solubilize the element

Table V Concentrations (µg kg^{-1}) of elements in the plankton of Kejimkujik, Pebbleloggitch and Beaverskin Lakes (σ = standard deviation; n = number of analyses)

Element	Kejimkujik			Pebbleloggitch		Beaverskin	
	Mean	σ	n	Mean	n	Mean	n
Al	11	1	7	9.8	4	7.8	4
Ca	110	15	7	82	4	72	4
Co	.7	.2	5	.8	3	.9	1
Cr	.2	.1	6	.1	4	.2	4
Cu	1.9	2.3	7	2.6	4	1	4
Fe	25	4	7	18	4	17	4
Hg(µg kg^{-1})	7	2	7	10	4	8	4
K	91	27	7	190	4	160	4
Mg	40	7	7	43	4	34	4
Mn	1.4	.3	7	.6	4	1	4
Na	76	13	7	100	4	95	4
Ni				.2	4	.2	2
P	320	70	7	360	4	250	4
Sr				.2	4	.2	4
Ti				.4	4	.3	4
V				.1	4	.1	4
Zn				10	4	5.2	4

from these compartments (Freedman and Hutchinson, 1986; Havas, 1986). Of the 3 lakes under study, the highest mean concentration of Al in water occurred in the most acidic lake (Pebbleloggitch; pH 4.3, .19 mg kg^{-1}, Table III), whilst the smallest concentration was in the most alkaline lake (Beaverskin; pH 5.3, .05 mg kg^{-1}, Table III). Aluminium concentrations in sediments (15.4 - 85.6 mg g^{-1}, Table IV) and in biota (3 - 860 mg kg^{-1}, Tables IV - VII) were present in much larger concentrations.

Mercury in fish The Hg concentrations in water (.07 - .1 mg kg^{-1}) were lower than the recommended limit for protection of aquatic wildlife (.2 mg kg^{-1}; CCREM, 1987). The maximum observed concentration of Hg in *Notemigonus crysoleucas* in this study was .2 mg kg^{-1} d.w. but the mean value, .15 mg kg^{-1} (n=18), corresponding to .038 mg kg^{-1} on a fresh weight basis (Bowen, 1979, p. 85), was much lower than the recommended upper limit for the protection of fish-eating birds and other wildlife (.5 mg kg^{-1} d.w.; International Joint Commission, 1977). Some other studies of mercury in fish of lakes in Eastern Canada have found higher concentrations than these, e.g. .3 - 1.3 mg kg^{-1} in 3 species in 11 lakes in N.W. Ontario; .1 - .4 mg kg^{-1} in 3 species in Lake of the Woods, Ontario; a mean of 2 mg kg^{-1} (maximum 5 mg kg^{-1}) in *Esox lucius* in northern Manitoba (Fimreite and Reynolds, 1973). Other studies have found similar mercury concentrations (e.g. mean values of <2 mg kg^{-1} in juvenile yellow perch of 10 Ontario lakes; Suns et al., 1980). However, Fimreite et al., (1973) reported analyses of relatively large and/or old fish of different species than the ones studied here. These and other factors are known to affect the mercury concentration of fish (Scheider et al., 1978; Suns et al., 1980; MacCrimmon et al., 1983). Water pH is another important factor, with acidic waters generally favouring the formation of bioavailable methylmercury compounds compared to dimethylmercury in less acidic waters (Tsai et al., 1975; Brouzes et al., 1977; Scheider et al., 1978).

All of the determinations reported are for total elemental concentrations, no analytical distinctions are made between e.g. ionic and organically bound forms. In terms of ecotoxicology, this is an important point, since ionic forms of toxic elements

Table VI Concentrations of elements in *Eriocaulon septangulare*, *Nuphar variegatum* and *Utricularia vulgaris* collected in Kejimkujik, Pebbleloggitch and Beaverskin Lakes (σ = standard deviation; n = number of analyses)

Element	Kejimkujik		Pebbleloggitch				Beaverskin		
	Eriocaulon	Nuphar	Eriocaulon	Nuphar		Utricularia	Eriocaulon		
Utricularia									
		mean	mean	mean	σ	mean	mean	mean	σ
	(n=1)	(n=4)	(n=5)	(n=8)		(n=5)	(n=4)	(n=7)	
(mg g^{-1})									
Al	.7	.2	.52	.09	.041	3.27	.52	5.65	.12
Ca	1.15	10.3	.38	12.3	1.1	3.97	.79	5.2	.3
Fe	3.4	.77	1.89	.49	.18	6.4	3.94	16.9	.7
K	4.9	10.3	1.14	10.8	1.3	.85	3.65	1.02	.11
Mg	.66	1.13	.34	1.4	.04	3.46	.46	2.73	.1
Mn	.225	.57	.044	.15	.004	.05	.3	3.54	.64
Na	5.34	4.85	1.71	5.98	.16	2.01	6.6	.656	.011
P	.83	2.43	.79	1.93	.1	1.65	.39	1	.03
Zn	.144	.024	.02	.02	.001	.064	.04	.076	.002
(μg 10g^{-1})									
Cd	7.5	11	8	11	1	10	20	24	1
Co	33	27	16	9	2	20	13	34	7
Cr	160	34	67	10	1	120	64	110	30
Cu	590	28	70	9	1	66	100	78	4
Hg	.09	.05	.09	.05	n=2	.18	.05	.27	n=2
Ni	31	35	33	20	2	75	44	91	3
Pb	120	26	260	140	20	540	210	460	50
Sr	68	710	51	380	60	420	79	540	30
Th	13		9			14	8	19	4
Ti	160	57	230	19	15	930	87	870	160
V	26	5	27	5	3	68	32	140	10
Zr	9	6	5			14	7	36	?

Table VII Element concentrations (mg kg^{-1} dry weight) in *Perca flavescens* (yellow perch) caught in Kejimkujik, Pebbleloggitch and Beaverskin Lakes (σ = standard deviation, n = number of analyses)

Element	Kejimkujik			Pebbleloggitch			Beaverskin		
	mean	σ	n	mean	σ	n	mean	σ	n
Al	6.1	3.4	11	3.3	1.9	9	17	6	6
Ca	13500	2100	14	11700	1900	20	11700	2500	18
Cr	.4	.2	14	.3	.1	20	.3	.1	18
Cu	.9	.4	14	.6	.2	20	.7	.3	18
Fe	21	7	14	17	3	20	35	4	18
K	2800	300	14	2440	310	20	2260	700	18
Mg	470	40	14	410	40	20	440	60	18
Mn	16	7	14	3	.8	20	18	11	18
Na	880	100	14	720	200	20	610	110	18
Ni	1.1	.6	12	1.1	.2	20	1.1	.8	18
P	8000	950	14	7010	980	20	7100	1200	18
Pb	.7	.2	14	2.4	.6	20	1.1	.2	18
Sr	29	13	14	33	7	20	32	7	18
Ti	.2	.1	8	.2	.1	17	.3	.3	12
Zn	24	6	14	17	2	20	23	6	18

are more readily bioavailable and toxic than are equivalent concentrations that are bound to dissolved organic materials or colloids such as suspended clays (Pagenkopf et al., 1974; Sunda and Lewis, 1978; Chau et al., 1974; Bowen, 1979; McKnight, 1981; Havas, 1986). Pebbleloggitch and Kejimkujik Lakes have large total concentrations of dissolved organic substances (Kerekes and Freedman, 1989) and these serve as complexing agents for metals, greatly reducing the relative concentration of the ionic fraction and thus the potential toxic effects.

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MORPHOLOGY, GROWTH AND PHENOLOGY OF *Petrocelis middendorffii*[†]

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The non-calcarious red alga *Petrocelis middendorffii* was studied *in situ* in the Bay of Fundy over the period October 1975 - May 1989. This species, widespread throughout the Bay, is found attached to rocks below the *Ascophyllum* zone, downwards to just beyond the *Mastocarpus-Chondrus* zone. In some sites e.g. Gulliver's Cove and Sandy Cove, Digby Co., the crusts occur only in a narrow zone about 1 m above mean low water between the *Mastocarpus-Condrus* and coralline zones. The diameter of crusts varied (0.01-0.5 m), and were generally thicker at the periphery than at the center. The thickness of the perithallium varied with season, the position in the crust, and the presence of developing tetrasporangia. Measurements in permanent quadrats revealed that crusts grew with a radial expansion rate of 1-1.5 mm y^{-1}. This growth occurred from spring to early autumn, and produced a pattern of concentric grooves that may be interpreted as annual growth rings. A coalescence of small crusts into a larger patch of crust exhibiting several concentric grooves was sometimes seen. Tetrasporangia were observed throughout the year. Mature tetraspores arranged cruciately were observed in intercalary perithallial filaments from late November to early March. Following the release of tetraspores during this period, the crusts turned greenish, except at the margins, and the discolored perithallial filaments were regenerated. After more than three years of monthly observations at Gulliver's Cove, we had not detected the germination of sporelings of either *Petrocelis* or *Mastocarpus* on substrates of cleared rocks, glass slides or permanent quadrats. However, *Ulva, Porphyra, Chondrus* and other macrophytes became established from spores at these sites.

On a éxaminé l'algue rouge non-calcaire *Petrocelis middendorfii in situ* dans la Baie de Fundy du mois d'octobre 1975 jusqu'à mai 1989. Cette espéce, largement répandue dans la Baie, s'attache aux roches, en bas du zone *Ascophyllum*, et en sa descente, dépasse le zone *Mastocarpus-Condrus*. Dans quelques lieux, p.ex. Gulliver's Cove et Sandy Cove, comté de Digby, les croûtes se trouvent seulement dans un zone étroit, à peu prés un mètre au dessus de la marée basse moyenne, entre les zones *Mastocarpus-Chondrus* et corallin. Les croûtes avaient un diamètre variable (0.01-0.5 m), et étaient ordinairement plus épaisses à la périphérie qu'au centre. L'épaisseure du périthallium changeait selon la saison, sa position dans la croûte, et la présence de tetrasporangia en développement. Les mesures faites en carats permenents ont démontré la croissance des croûtes en expansion radiale à un taux de 1.0-1.5 mm chaque année. Cette croissance prenait place du printemps jusqu'à la première partie de l'automne, et produisait une disposition de sillons qu'on peut interpréter comme anneaux de croissance. Un groupement de petites croûtes produisant une plus grande parcelle de croûte et démontrant plusieurs sillons concentriques a été décerné de temps à autre. On a découvert des tetrasporangia au long de l'année. Des tetraspores mûrs et cruciformes ont été décelés dans les filaments intercalaires du périthallium, depuis la dernière partie de novembre jusqu'à la premiere partie de mars. Apres l'émission de tetraspores, les croûtes sont devenues verdâtres excepté aux marges, et le périthallium décoloré s'est érodé de la surface de la croûte. Par la suite les filaments périthalliales se sont dégénérés. Pendant plus de trois années d'observations mensuelles à Gulliver's Cove, nous n'avons pas aperçu la germination de jeunes spores soit de *Petrocelis* ou de *Mastocarpus* sur les souches inférieures de roches deblayées, de plaques de verre, ou de cadrats permanents. Toutefois *Ulva, Porphyra, Chondrus* et d'autres macrophytes se sont établis en provenance de spores sur ces lieux.

Introduction

Petrocelis middendorffii (Rupr.) Kjellman is commonly found in the Bay of Fundy, Nova Scotia (Edelstein *et al.*, 1970; Wilson *et al.*, 1979; South, 1984) associated with an abundance of *Mastocarpus stellatus* (Stackh. in With.) [Guiry et al., 1984; = *Gigartina stellata* (Stackh. in With.) J. Ag.] *M. stellatus* is also very common along the Atlantic coastal waters of Nova Scotia, but *P. middendorffii* is extremely rare (Edelstein *et al.*, 1970).

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West (1972) suggested that *P. middendorffii* or *Petrocelis cruenta* J. Agardh could be the tetrasporophyte of *M. stellatus,* but our early work on *M. stellatus* carpogonia (Edelstein *et al.,* 1974, as *Gigartina*) from the coastal waters of Nova Scotia and plant development in culture from carpospores (Chen *et al.,* 1974) did not indicate a linkage between these taxa in our area. Evidence, such as observed for *P. franciscana* Setchell et Gardner, (West, 1972) and *P. cruenta* (West *et al.,* 1977; Dion and Delépine, 1979), of alternation in the life history between foliose *Mastocarpus* and crustose *Petrocelis* was not seen. A number of studies have shown that heteromorphic and apomictic life histories occur in the *Mastocarpus (Gigartina)* species from the Pacific coast of Japan (Masuda *et al.,* 1987), California and Washington (West *et al.,* 1977), the Atlantic coasts of France (Dion and Delépine, 1979) the British Isles, Portugal, Spain and Denmark (Guiry and West, 1983). However, the life history of *M. stellatus* appeared to be exclusively apomictic in Nova Scotian waters (Chen *et al.,* 1974) and in Norway (Rueness, 1978). In 1981, Chen and Craigie (1981) reported on cultured *M. stellatus* plants with basal holdfasts that increased greatly in size over ten years. Analysis showed that these holdfasts contained λ-type carrageenan, the type of carrageenan found in field-collected *Petrocelis* plants and different from that produced in the apomeiotically reproducing erect fronds.

A study (Paine *et al.,* 1979) on growth and longevity of *P. middendorffii* in the coastal waters of Washington reported that this crustose red alga exhibited slow growth, a relatively great longevity and persistence. However, the report has shed little light on the mechanism(s) of persistence, but suggested that the crustose algal periphery might be less edible to molluscan grazers than the central portion, which has excellent capacity to re-establish itself. The report did not indicate that there was any evidence of linkage between *G. papillata* and *P. middendorffii* in that region. Guiry and West (1983) reported comprehensively on the linkage between *M. stellatus (G. stellata)* and *P. cruenta* which cover wide regions of the Atlantic coasts. However, they gave little information concerning *P. cruenta* in the Maritimes. Recently, Dudgeon *et al.* (1989, 1990) reported *Mastocarpus stellatus* to be a very hardy species with regard to freezing tolerance in the coastal waters of Maine. *Petrocelis* is very common in the Bay of Fundy, yet little is known regarding its morphology, growth, regeneration, colonization, seasonal variation or reproduction. We report here a long-term study of these aspects of *P. middendorffii* from the Bay of Fundy.

Materials and Methods

Field observations on a montly basis were made from October 1975 to May 1989 along the shores of the Bay of Fundy and occasionally on the Atlantic coastal waters of Nova Scotia (Fig 1). Twelve permanent quadrats were randomly established in the littoral zone at Gulliver's Cove, Digby Co., by chiselling into the rock a 1 dm square perimeter. Each quadrat was marked with fluorescent paint along the chiselled line so that the quadrats could be relocated. Quadrats were photographed and *Petrocelis* crusts and any other macroalgae counted. The elevation and position in the littoral zone of each quadrat (Fig 2) were recorded.

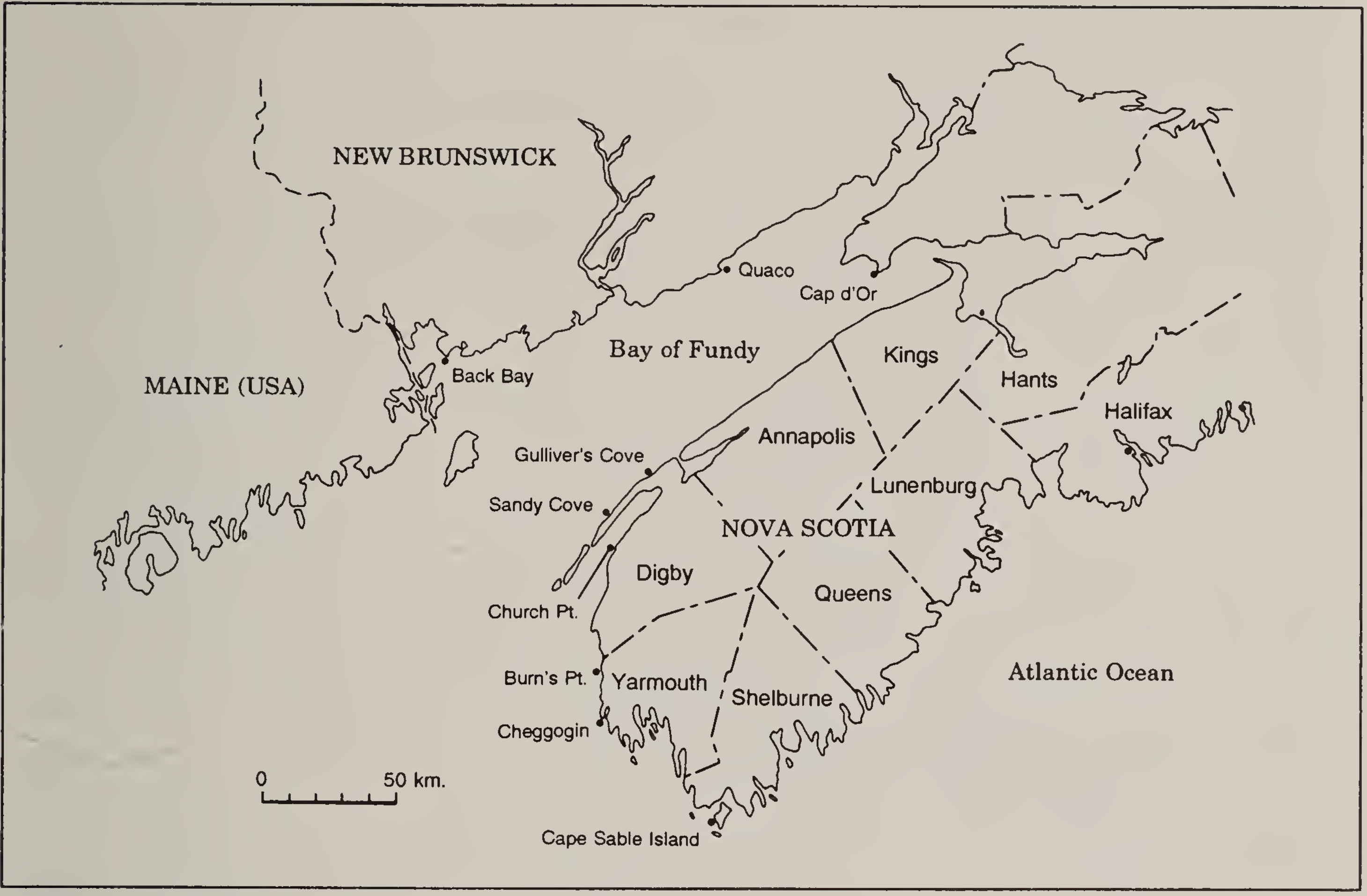

Fig 1 Study sites in the Bay of Fundy.

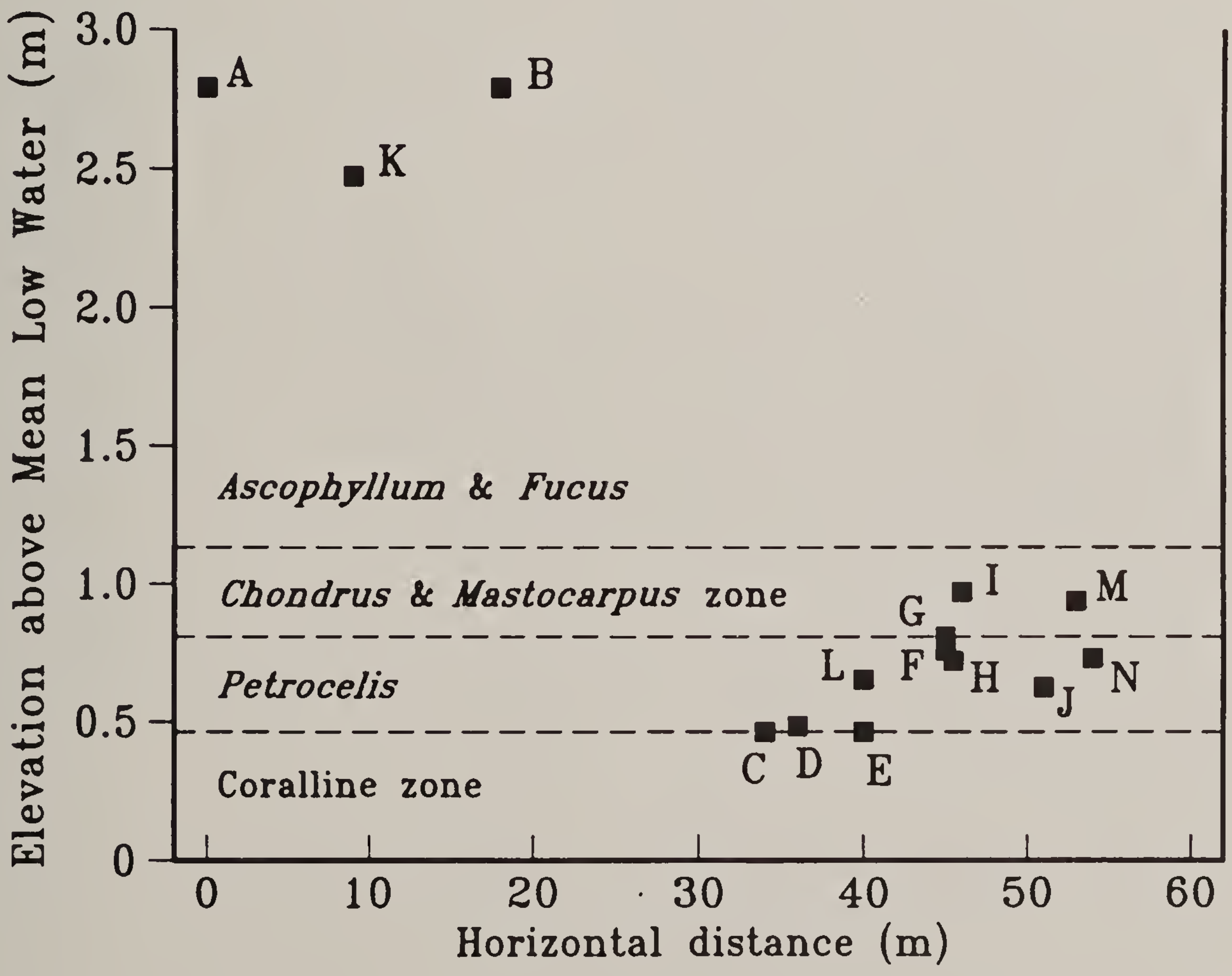

Fig 2 Distribution sample plots (quadrats) in the intertidal zone at Gulliver's Cove.

Table I Change in area of *Petrocelis* crusts as determinated in permanent quadrats at Gulliver's Cove*

	Area (cm²)		
Quadrat**	November 1975	November 1976	January 1978
C	2.5	4.1	5.3
D	2.0	3.8	4.5
E	4.4	6.5	7.8
F	1.9	3.0	2.8*
G	4.4	7.4	9.0
H	0.9	1.7	2.5
I	2.3	3.2	5.2
J	2.4	5.2	6.2
M	8.8	8.3*	10.9
N	15.3	20.0	22.8

* Decrease in crust following spore release.
** A & B had no *Petrocelis* crusts.

One of the quadrats (H) was set up to study *Petrocelis* thallus regeneration capability over the period from 1976 to 1979. Initially, we removed approxiamtely two cm^2 (1 x 2 cm) from two locations on the crust at the margin area. At the first location both the hypothallium and perithallium were completely removed by using a surgical blade, whereas at the other one, only the perithallium was sheared off. Samples from the same study sites were taken monthly thereafter and were examined microscopically.

Monthly examinations and photographs of quadrats were made at low tide from 1975 to 1978 (Table I). For each quadrat, horizontal growth of crusts, regeneration, recolonization, population changes, reproductive maturity, animal grazing, recruitment and mortality were assessed.

Petrocelis crusts were also collected from outside the quadrats at Gulliver's Cove and from other locations in the Bay of Fundy to monitor the anatomical structure, initiation of tetrasporangia, release of tetraspores, regeneration of perithallium, and the proportion of perithallium to hypothallium. Entire small crusts were scraped from the rock to determine the developmental pattern of perithallium and hypothallium.

To observe succession and recolonization of *Petrocelis* in nature, the algal population on two boulders, one just below the *Ascophyllum* zone, the other lower, at the *Mastocarpus-Chondrus* level at Gulliver's Cove (Fig 2, K & L) were cleared of all attached plants with a kerosene flame thrower (Edelstein *et al.*, 1970) in October, 1975. Simultaneously, two sets of ground glass slides (10 each) were set up near each boulder as portable substrata for recruitment of sporelings. The boulders and slides were observed monthly for one year, and observation of the boulders continued periodically from 1977 to 1989.

To study growth rates, the *Petrocelis* crusts in each quadrat were photographed *in situ*, at one to three-month intervals over a period of thirty months beginning in September 1975. Thereafter, photographs were taken twice a year until 1989. All photographs were taken from approximately the same angle and distance from the quadrat to minimize problems of parallax. Photographs were printed to the same scale to facilitate comparison of the measurement of growth and horizontal expansion of the crusts during the observation period.

Observations

Distribution, habitat and associated species - *Petrocelis* was abundant on rocks at the collection sites in the Bay of Fundy except for the uppermost portion (beyond Quaco) of the Bay (Fig 1), where populations were sparse. The vertical distribution of *Petrocelis* varied throughout the Bay. On the New Brunswick or northwest coast, from Quaco Head westward toward St. Andrews, patches of *Petrocelis* (up to 30 cm in diameter) were common especially in the mid-intertidal zone. Crusts (>20 crusts m^{-2}) were abundant in the *Ascophyllum* zone, attached to the same rocks as the *Ascophyllum* plants, but *Petrocelis* was also well developed slightly below the *Ascophyllum* zone. However, on the Nova Scotia side of the southeastern shores of the Bay of Fundy, these crusts were found only in the low intertidal zone in various places, such as Cape d'Or, Cumberland Co.; Gulliver's Cove, Sandy Cove, Tommy Beach and Church Point, Digby Co.; Burn's Point and Chegoggin, Yarmouth Co. In these locations, patches of crusts < 1 - 50 cm in diameter were found below the *Ascophyllum* zone and growing between the *Mastocarpus-Chondrus* and *Laminaria* zones. Foliose *Mastocarpus* plants were always present wherever *Petrocelis* crusts were found. Curiously, the situation was quite different in coastal waters from Cape Sable Island (Yarmouth Co.) to Fink Cove, Halifax Co. where foliose *Mastocarpus* plants were common in the intertidal zone without any sign of crusts of *Petrocelis.*

Petrocelis was very common at Gulliver's Cove and some crusts were up to 50 cm or more in diameter. Both the small crusts and some of the large patches were usually round with very distinctive concentric grooves on the surface. We also found crusts with several sets of concentric ridges on a single large patch of crust. The shape of the crusts was usually governed by the underlying substrate to which the crusts were attached.

At Gulliver's Cove and Sandy Cove, *Petrocelis* crusts grew in a narrow zone approximately one meter above the mean low water mark (Fig 2), usually between the *Mastocarpus-Chondrus* and coralline zones. The crusts were sometimes found below and adjacent to the *Ascophyllum* zone but not above it (Fig 2, A & B have no *Petrocelis* crusts). These crusts usually grew together with the morphologically similar crusts of *Hildenbrandia rubra* (Sommerf.) Menegh. During the spring season both appeared reddish-brown in colour and were firmly attached to the rock-substrate. However, they could be distinguished macroscopically later in the year, when the *Petrocelis* crusts became thicker, darker and showed concentric zones; such zones were absent in the *Hildenbrandia* crusts, which remained thin and became a lighter reddish brown.

Examination of randomly collected samples of the crusts indicated that these species grew intermixed down to the coralline zone, and furthermore, that *Hildenbrandia* occurred largely at the upper edge of the mid-intertidal zone and gradually diminished towards the lower intertidal zone (*Laminaria* zone). *Petrocelis* crusts (5-10 cm in diameter) were abundant (< 20 crusts m^{-2}) in the *Mastocarpus-Chondrus* zone, but most abundant (> 20 crusts m^{-2}) below the *Mastocarpus-Chondrus* zone on vertical boulders facing the open water. However, below the *Laminaria* zone (in the infralittoral fringe) these crusts disappeared.

At most of our collection sites in the Bay of Fundy, *Petrocelis* crusts competed for space with other crustose algae, such as *Ralfsia fungiformis* (Gunner) Setchell et Gardner, *Peyssonnelia rosenvingii* Schm. in Rosenv. and especially coralline red algae. The last were often found to overlap with *Petrocelis.* At the low water level among the *Alaria* and *Agarum,* large boulders were occasionally found to be covered with extensive patches of smooth, dark reddish-brown *Peyssonnelia* crusts.

Animals such as limpets and marine snails were common throughout the year on the surface of *Petrocelis* crusts, except in January, when they were noticeably rare.

Seasonal aspects - Many changes in the colour of *Petrocelis crusts* were observed throughout the year. In general, the colour was dark reddish-brown for both *Mastocarpus* and *Petrocelis,* but darkest and richest in December when the illumination was lowest and growth was reduced. Anatomical observation showed tetrasporangia were abundant in this month. In late January some greenish spots appeared on the surface of the crusts, in some cases expanding over the whole surface except the peripheral area. By March, the surface colour of many crusts has gradually returned to light brown and reddish-brown. Observations confirmed that tetraspores had been released from the filaments during this period. Deterioration of tissue during and after release of tetraspores might have contributed to the change in colour of the crust surface. From April to August many portions of the *Petrocelis* crust appeared somewhat yellowish, particularly in the central zones. This colour change may have also been related to the amount of light received.

Anatomy and physical characteristics - The crustose thallus of *P. middendorffii* consists of a basal, compact layer of multicellular hypothallium bearing an upper layer of relatively loose erect perithallial filaments. The hypothallium is initially established as a radially filamentous layer which expands horizontally to increase the size of the crust and forms upward-growing loosely coalescent filaments to develop the upper hypothallium and perithallium. The thickness of the cursts can vary from 0.49 to 1.75 mm.

The hypothallial layer consisted of irregularly shaped cells that had long cross links to laterally adjacent cells by pit connections. This tissue sometimes also had a very compact lower stratum of cells loosely connected to the upper portion of the layer. The range in thickness of the hypothallium was 0.24 - 1.00 mm. The perithallial layer consisted of rectangular cells (5-10 x 11-25 µm) in loosely cohesive erect filaments. Generally, the cells of the perithallium were linked only by primary pit connections, with an occasional lateral secondary pit connection in the lower portion close to the hypothallium. Perithallial filaments were branched dichotomously, usually in the upper third of the tissue layer; however, dichotomies occasionally occured quite close to the hypothallium. In some areas of the perithallium, the cells were more irregular and compact, and had more secondary pit connections which apparently caused the distortion in shape. The thickness of the perithallial layer varied from 0.25 - 0.91 mm.

The thickness of the hypothallium and the perithallium appears to depend upon the season, position on the crust, stage in reproduction, growth and age. For example, at the crust margin the hypothallium normally is slightly thicker than the perithallium; however, in early spring the perithallium was thicker. In addition, during the period of the formation of tetrasporangia and before tetraspore release, the thickness of the perithallium increased significantly. Samples of young crusts of *Petrocelis* showed that the marginal areas were always thicker than the central region.

The mean thickness of crusts examined was thinner in May than in August; this might be a result of the intercalary perithallium growing more rapidly during the summer. The ratio of hypothallium to perithallium thickness varied from 0.54 to 1.5, in monthly samples collected from quadrats from April to December. This variability might be explained by the factors influencing the thickness of the crust mentioned above. As well, samples from the extreme outer zone of *Petrocelis* crusts showed a variety of growth patterns. In some cases they had a well-developed hypothallium with short perithallial filaments and very actively growing apical cells. There were also some long perithallial filaments and sometimes apparently a single layer of large irregular hypothallial cells. These growth patterns might indicate that the crust had begun to expand horizontally.

A small green *Codiolum*-type alga, ("*C. petrocelidis*"), was commonly found growing between the filaments of the perithallial layer of *Petrocelis,* especially near the margins. Only a few young *Codiolum* plants were found in the hypothallial layers. This endophytic alga did not seem to affect the development of *Petrocelis* crusts.

Tetrasporocytes were found monthly throughout the year, occurring irregularly in small patches on the crusts. Mature divided sporangia, 40-50 x 20-30 µm in diameter, were found only from late November to February; the mature spores were arranged cruciately in the sporangia and were ready for discharge. Discharged tetraspores measured approximately 20-30 µm in diameter. There was generally a single intercalary tetrasporangium per filament of perithallium. However, in a few exceptional cases there appeared to be more than one developing sporangium per filament, arranged in a series as Wilce & Maggs (1989) described for *Haemecharia hennedyi* comb. nov.

The location of tetrasporangia on perithallial filaments can vary, sometimes being very near the apex, sometimes close to the hypothallium. Following release of tetraspores, patches on the surface of crusts (except near the edges) turned greenish and later eroded. This phenomenon was prevalent in early March; thereafter, the crusts gradually recovered their original reddish-brown colour. By April, the number of tetrasporangia in the samples had decreased dramatically and they were observed only occasionally after April. In general, our observations were in agreement with those of Polanshek and West (1975, 1977) on crusts of *P. franciscana.*

Recruitment and regeneration - At the two quadrats (Fig 2, K & L) set up in Gulliver's Cove to study colonization and succession, populations of *Petrocelis* and other algae eventually re-established themselves. However, the manner in which this occurred in each of the two sites appeared to be different. Monthly observations during the first year showed no signs of *Petrocelis* crusts on the boulder (K) in the *Ascophyllum* zone. However, *Fucus vesiculosus L., Ascophyllum nodosum (L.) Le Jol, M. stellatus, (G. stellata), C. crispus, H. rubra* and encrusting corallines had regenerated there. Interestingly, on the boulder (L) located at the *Mastocarpus-Chondrus* level, *Petrocelis* crusts were found developing mainly along cracks in the rock. There were also a few seasonal plants such as *Porphyra miniata, Ulva lactuca, Polysiphonia urceolata* and *Petalonia fascia.* On neither of these boulders nor the glass slides did we see any young sporeling crusts of *Petrocelis, M.* stellatus, *C. crispus,* or *Fucus* spp. However, some marine animals including snails and limpets were found.

During the periodic observations from 1977 to 1989, we found crusts of *Corallina officinalis* and *H. rubra,* growing on both of the boulders along with *Fucus evanescens C. Ag., C. crispus* and other macrophytes. On the boulder in the *Mastocarpus-Chondrus* zone, the crusts of *Petrocelis* found initially along the cracks of the rock, gradually expanded in size. There were also two young *M. stellatus* plants developing along the cracks in this rock. Three possible explanations for this are: germination from tetraspores of *Petrocelis;* germination from the carpospores of *M. stellatus;* and vegetative regeneration from parts of holdfasts that may have survived. However, the glass slides set up near the boulders to collect germinating plants showed no crustose algae had colonized there after one year. Some annual plants such as *Porphyra, Polysiphonia,* and unidentified green and brown algal sporelings were found on the slides. This parallel recolonization experiment suggests that it is unlikely that the reappearance of crusts and young *Mastocarpus* plants along the rock cracks resulted from germination of spores.

Our regeneration studies were carried out on a fixed quadrat from June to October 1976. During this period there was little indication of regeneration after both hypothallium and perithallium of the crusts had been removed from the substrate. After 36 months some regeneration was noted, presumably from microscopic residual tissue. By contrast, in the crusts where only the perithallium was removed, some regneration of perithallial filaments occurred after two months. In cases where parts of the hypothallium were damaged as the perithallium was scraped away, the crust became yellowish and began to degenerate.

Horizontal growth - Table I shows the growth of individual crusts measured in each quadrat (except A & B) from 1975 to 1978. Overall, the crusts exhibited slow expansion (Table I). Some of these crusts however, decreased in area due to erosion of a certain portion of the crusts. In these cases, some marine animals on the crusts of *Petrocelis* were observed.

In one of the quadrats in the horizontal expansion study there were four small crusts which we observed for long-term growth. The individual crusts grew very slowly, widening in radius at a rate of 1-1.5 mm a^{-1}. Fig 3 represents diagrammatically the

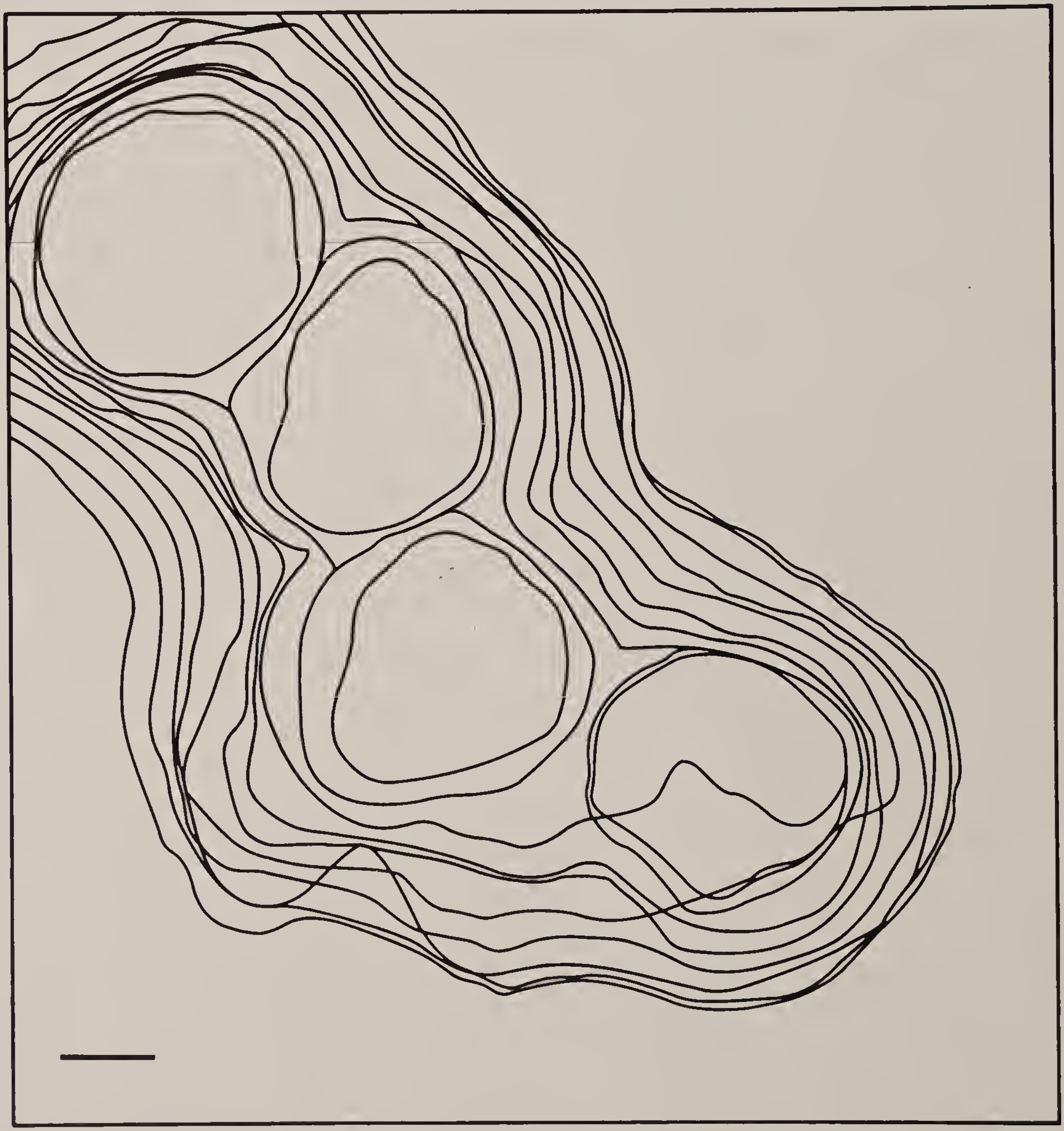

Fig 3 A diagrammatic representation of the horizontal expansion of four crusts during a 12 year period. Each line represents yearly expansion.

horizontal expansion of the crusts during a 12-year period. Successive area expansions were drawn from photographs and these drawings were superimposed showing how both the area and the shape of the crusts changed with time (as in *Chondrus crispus* holdfasts, Taylor *et al.*, 1981). The diagram shows that some overlapping and merging of crusts took place.

The observations recorded for each quadrat in the horizontal expansion study indicated that there were some tetrasporangia and changes in colour of crusts. Most of the crusts that were selected initially were small individuals, but after a few years they began to merge with one another (Fig 3). During this period, there was no indication of new *Petrocelis* sporelings or new crusts. However, some annual algae were observed in these quadrats.

Discussion

Our observations show that *P. middendorffii* is a perennial plant with an extremely slow horizontal expansion (Table I), capable of attaining great age and size under favourable environmental conditions. The regeneration studies in which crust tissue was partially removed indicate that these crusts have an excellent capacity to regenerate to their original form from portions of filaments retained in the rock matrix. They are surprisingly capable of recovering within two months after damage to the perithallial filaments. In other cases where both perithallial and hypothallial filaments were damaged, they may take years to recover. This evident persistence suggests that the population of crusts once established may remain on a substrate so long as the substrate exists. However, growth of *Petrocelis* crusts was restricted by competition for space with other crustose algae on the substrate. The regeneration investigation shed little light on recruitment of original crusts, in spite of the abundance of tetraspores of *Petrocelis* and carpospores of *Mastocarpus* in this area. Nor was there any evidence of sporeling recolonization *in situ* on the glass slides, on the cleaned rock surfaces or on the permanent quadrats. One possible explanation is that because of the apparent vulnerability of the released spores to the severe environmental stresses in the study area, germination was unable to occur. Overgrazing by a large population of animals may have also contributed to the lack of recruitment or re-establishment of sporeling by *Petrocelis* during the course of this study. Further laboratory study might lead to better understanding of the germination capability of tetraspores of *Petrocelis* and carpospores of *Mastocarpus (Gigartina)*.

Our observations suggest that overall size of the crust is not necessarily an indication of age. In some cases, large patches of crusts of *Petrocelis* observed in nature appear to be the result of many small crusts merging with one another as shown in Fig 3. Throughout the collection sites, single large crusts were often found to have several intersecting sets of concentric zones. This suggests that several small crusts had merged to form a large single patch of crust. Figure 3 shows the growth of four small individual crusts, which changed their shape and size from year to year until they finally merged as indicated by four sets of concentric grooves on one large patch.

We agree with the previous report (Paine *et al.* 1979) that regeneration of damaged central portions of the crusts results in slow expansive growth. However, we disagree with their explanation that the cause of this damage was by grazing molluscs which find the central perithallial tissue to be more palatable than the peripheral portions of the crust. Our observations show that damage to the perithallial tissue is a natural part of the reproductive process. Investigation of seasonal colour changes on the surface of the crusts led to the observation that the release of tetraspores weakens perithallial tissue causing portions of the tissue to erode. Severe environmental conditions, such as sub-zero air temperatures at low tide during the reproductive season may exacerbate this damage to the perithallial tissue.

Measurements of thallus thickness and ratio of the length of perithallial to hypothallial filaments were relatively uniform among very small crusts collected from October to early December, just before the tetraspores were discharged but the samples collected randomly from December to September showed great variation. The seasonal variation in thickness of crusts might also be related to the development of reproductive structures. Localized erosion of perithallial filaments after release of tetraspores caused changes to both the overall thickness and ratio of thickness between the two layers. The concentric zones in the crusts would further contribute to this localized variability. It appears that both the variation of thickness and ratio of the length of perithallium to hypothallium depend upon season, the reproductive period of the crust, the location on the crust where the measurement is taken, and especially, whether or not tetraspores have been discharged. Thus, the overall thickness of crust and the ratio of the hypothallium to the perithallium may not be useful characteristics to distinguish taxa. Table II summarizes the morphological characteristics of northeast Pacific *P. middendorffii*/*P. franciscana, P. cruenta* from Ireland and *P. middendorffii* in the Bay of Fundy. It is apparent that the anatomical features of these crustose algae show great variability, making the crusts indistinguishable on morphological grounds. We feel that further comparative culture and cytological studies among strains from typical localities throughout the know ranges are needed in order to use these characteristics to clarify and distinguish the different types of crusts. It is possible that analysis of nucleotide sequence data, may also be used as a taxonomic tool.

The life history studies of *Mastocarpus (Gigartina)* species from various regions (West, 1972; Polanshek and West 1975; West *et al.*, 1978; Dion and Delépine, 1979;

Table II Summary of morphological characteristics of *Petrocelis* crusts from various locations.

Species:	*P. middendorffii** *P. franciscana*	*P. cruenta**	*P. middendorffii*
	Location		
Characteristics	North Pacific	Waterford, Ireland	Bay of Fundy
Perithallium (thickness)	200-900 µm	400 µm	250-1000 µm
Hypothallium	50-175 µm	300-400 µm	150-540 µm
Crust (total thickness)	0.25-1.1 mm	0.7-0.8 mm	0.5 mm-1.3 mm
Tetrasporangia	17-30 x 25-35 µm		20-30 x 30-40 µm
Tetraspore diameter	14.6-16 µm	13 µm	12-20 µm
Rectangular cell of the perithallium	3-4 x 8-14 µm		5-10 µm x 11-20 µm
A phase of	*Mastocarpus papillatus*	*M. stallatus*	?

* West *et al.* 1977.

Masuda and Kurogi, 1981; Guiry and West 1983; Masuda *et al.*, 1987) indicate that *Petrocelis* is the tetrasporic phase of *Mastocarpus* (formerly a subgenus of *Gigartina*). West *et al.* (1978) demonstrated that *P. middendorffii* in Pacific coastal waters is the tetrasporic phase of *G. papillata (M. papillatus)*, a species which has not been reported in the coastal waters of Nova Scotia. Obviously, the so-called *P. middendorffii* in this region is different from the Pacific coastal plant. Moreover, from our field observations in the Bay of Fundy, there is no positive evidence to suggest that *P. middendorffii* alternates with *M. stellatus*, despite the fact that the latter is commonly found in association with *Petrocelis* in the Bay of Fundy. It is also interesting to note that *M. stellatus* is abundant in the Atlantic coastal waters of Nova Scotia where no *Petrocelis* was found during this study. Whether or not *M. stellatus (G. stellata)*, which is reported as the gametophytic phase of *P. cruenta* of northeastern Atlantic coastal waters (West *et al.*, 1977), is an alternate generation of "*P. middendorffii*" in Nova Scotia coastal waters has to await further study for clarification. At present, we are unable to demonstrate unambiguously a linkage between local *Mastocarpus* and *Petrocelis.*

We were unsuccessful in observing the recruitment of *Petrocelis* in nature. In the cleared natural substrate monitored from 1975-1978, the glass slides set up *in situ* for one year, and the twelve permanent quadrats observed over nearly 14 years, there was no evidence of sporeling germination or colonization. We saw neither the tetraspores of *Petrocelis*, nor the carpospores of *M. stellatus* germinating on these substrates, although some macrophytic algae did re-establish themselves on the burned boulders and the permanent quadrats after several years. We did observe some crusts of *Petrocelis* developing along cracked areas in these boulders which probably indicates that these *Petrocelis* crusts may have regenerated from those portions of the tissue retained in the rock metrix after animals grazed (Paine *et al.*, 1979); or perhaps the natural degeneration of cells from the filamentous portion of the crusts left isolated groups of cells on the boulders from which the new crusts regenerated. It may also be that these crusts in the deeper cracks had not been completely removed, or perhaps the rough surface in the deeper cracks had trapped the released spores thus allowing vegetative regeneration of new populations of *Petrocelis.* We have no conclusive evidence to show that patches of *Petrocelis* along the sides of rock cracks were the result of germination of the carpospores of *M. stellatus.* Only once, during the period of our studies, did we detect some small crusts in the vicinity of these denuded boulders, which had also formed on a few other boulders which we suspect may have developed from spores. They may also have developed from small amounts of tissue remaining after animal grazing. Further studies on the viability and germination of the tetraspores from *Petrocelis* are needed in order to develop a comprehensive understanding of the question of linkage between *P. middendorffii* and *M. stellatus.*

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Among many reasons for these expansions of the Institute's publishing activities is the realization that many scientists, during their careers, have accumulated valuable databases pertaining to their scientific interests. This information is of great value to others, but whilst much of it can be recovered from the literature such recovery is a time consuming and tedious job. By publishing such specialized databases in electronic format (and passing on appropriate royalties to those who have created them), we hope that the Institute will serve a useful purpose.

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RECOMMENDATIONS AUX AUTEURS

La Revue *Proceedings of the Nova Scotian Institute of Science* publie les travaux de l'Institut, des articles originaux et des notes avant trait aux sciences naturelles aux Provinces atlantiques, surtout dans les domaines de la biologie et de la géologie. Les manuscrits peuvent être en anglais ou en français, et seront soumis à l'avis d'experts avant d'être acceptés.

Les textes doivent être dactylographiés à interligne double, sur des feuilles de papier blanc 21.5 cm sur 28 cm (8.5 po sur 11 po), avec marges de 4 cm (1.5 po). Ne souligner que les passages destinés à être imprimés en italique; utiliser les majuscules dans le seul cas où les lettres ou les mots en question doivent paraître en majuscules à l'impression. La mise en page et la typographie suivront le modèle du présent ouvrage. Le texte original doit être accompagné d'une copie. Chaque page doit être numéroté, la première page ne portant que le titre, le nom des auteurs, la mention de l'université ou autre établissement auquel ils sont affectés, et des notes infrapaginales éventuelles.

L'orthographe doit se conformer au **Larousse encyclopédique**. Les auteurs sont responsables de l'uniformité de l'orthographe dans leurs textes.

Les abréviations, la nomenclature et les symboles des unités de mesure doivent se conformer aux normes internationales. Les unités métriques (SI) et les décimales doivent être utilisées dans la mesure du possible. Pour les dates, adopter l'ordre jour/mois/année. Ne pas faire suivre d'un point les abréviations "mm, kg, ME, CNRC", etc.

Les tableaux taxonomiques doivent se présenter, dans des cas de la zoologie et de la paléontologie, sous forme de descriptions dichotomiques alignées verticalement, et pour la botanique sous forme multilinéaire étagée. La synonymie, en botanique, en zoologie et en paléontologie, doit être représentée succinctement: taxon, auteur, année, page, avec citation au complet aux indications bibliographiques.

Un résumé ne dépassant pas 200 mots doit précéder le corps du texte. Le texte lui-même doit comprendre les sections suivantes: introduction, méthodes, résultats ou observations, discussion, références bibliographiques. Dans certains cas, une systhèse de deux ou de plusieurs de ces sections peut étre souhaitable.

Les références bibliographiques doivent être vérifiées auprès des publications d'origine, et doivent être identifiées dans le texte par les mentions auteur et date, entre parenthèses. Les références bibliographiques doivent être arrangées par ordre alphabétique en fin de texte. Les références aux articles parus dans des revues doivent comporter les éléments suivants: nom du journal, numéro du volume, pagination complète. L'abréviation des titres revues doivent se conformer au **Bibliographic Guide for Editors and Authors** (American Chemical Company, 1974). Pour les revues qui n'y figurent pas, consultera la **World List of Scientific Periodicals** (Butterworths, 1963). Nous donnon ci-dessous quelques examples de référence aux revues, livres et recueils:

Auteur, U.N. et Autre, T.E.L. 1978. Titre de l'article. *Nom de la revue,* (volume) 99, 1-10. (Omettre le mot "volume").

Auteur, U.N. et Coauteur, S.O.N. 1978. *Titre du livre au complet.* Editeur, Ville.

Auteur, U.N. 1978. Titre de l'article. In *Titre du livre ou du Recueil.* (rèd. A. Lenoir et B. Leblanc) Editeur, Ville, pp. 256-301.

Les tableaux doivent être numérotés en chiffres romains, avoir un titre concis et être signalès dans le texte. Ils ne doivent pas comporter de lignes verticales. Les tableaux doivent être soumis sur feuilles à part.

Les dimensions de originaux des illustrations ne doivent pas dépasser le triple de celles de leur reproduction définitive, soit 11 cm sur 10 cm. Les figures, y compris celles des planches, seront numérotées en chiffres arabes, et doivent être signalées dans le texte. Les dessins originaux doivent être exécutés à l'encre de Chine sur du papier blanc. Tous les traits doivent être suffisamment épais pour en assurer la reproduction bien nette. Les lettres et autres caractères doivent être de dimensions à rester parfaitement lisibles à la réduction, et en aucun cas ne doivent être inférieurs en à l'état réduit, à 1 mm, hauteur. Les originaux des illustrations sont exigés, ainsi qu'un jeu de copies bien propres. Les légendes des illustrations doivent être ècrites à la machine sur feuille à part.

Les photographies doivent être montées sur du bristol bien rigide, prêtes à la reproduction. Les épreuves, sur papier glacé, doivent être d'excellent qualité. Les photographies destinées à être groupées doivent être montées ensemble sans intervalle. Les photographies de qualité inférieure seront refusées, ce qui risque d'entraîner des remaniements importants du manuscrit.

Les illustrations en couleur sont acceptables, à la discrétion de la rédaction. Toutefois, les frais de reproduction en couleur restent à la charge des auteurs, qui doivent d'ailleurs en accepter la responsabilité par ècrit.

Les auteurs seront responsables de la correction des épreuves. Les commandes de tirage à part, en sus des 15 fournis à titre gratuit, doivent être passées au moment de la remise des épreuves corrigées.

PROCEEDINGS
of the
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HALIFAX, NOVA, SCOTIA

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NOTICE ÉDITORIALE

Afin de rendre un service davantage aux membres qui font usage d'un ordinateur, le Conseil de l'Institute va aggrandir ses activités en publications par deux biais.

1. L'Institut va publier certains sujets encyclopédiques en format électronique ce qui permettra à nos membres de s'en servir sur leurs ordinateurs. Par exemple, cette Partie du Volume 39 des Comptes-rendus fournira un index compréhensif d'Auteurs et de Sujets des Volumes 1 à 39. Cette partie sera disponible dans la forme imprimée habituelle ainsi que sur des disques magnétiques (3.5 pcs. et 5.25 pcs.) qui sont lisibles sur des dispositifs Macintosh et IBM (DOS). Le Conseil prévoit un taux de faveur aux membres qui vondraient acheter ces données de base.

2. Dans chaque publication des Comptes-rendus, l'Institut a pour but l'inclusion d'une colonne intitulée "Sujets Turing" (en honneur du célèbre mathématicien de ce nom). Nous invitons no lecteurs à partager, par quelque phrases, leurs aventures (bonnes ou mauvaises), basées sur l'abondance de produits logiciels qu'ils ont en main. Nous espérons que ceci servira de guide pour tous les hommes de science. De plus, cette colonne présentera les noms, les adresses des vendeurs, et le prix des matériaux qui viennent à notre connaissance. L'apparition de ces renseignements **ne suggèrent aucunement** l'approbation des produits; un choix quant à l'utilité de ces produits pour nos membres sera achevé conscientieusement. La colonne renfermera une liste des produits mentionnés ci-dessus. Cette liste donnera le titre, un abrégé des renseignements, ainsi que les prix.

Pour plusieurs raisons, nous avons été conduits à l'expansion des activités de publication de l'Institut, entre autres, nous sommes devenus convaincus que plusieurs hommes de science, lors de leurs carrières professionelles, ont amassé des données de base apparentées à leurs intérêts scientifiques. De tels renseignements sont de grande intérêt et de valeur à d'autres savants, car, malgré qu'il soit possible de dénicher cette information dans les journaux scientifiques, une telle démarche demande beaucoup de temps, et devient très ennuyeuse. La publication de données de base spécialisées, en format électroniques, remplira, à notre avis, une fonction utilitaire. (Nous espérons aussi que les droits d'auteur justifiables seront remboursés à ceux qui ont créé ces produits).

PROC. N.S. INST. SCI. (1980)
Volume 39, pp. 149-247

Cumulative Author and Subject Indices

to proceedings of the

Nova Scotian Institute of Science

Volumes 1-39 (1863 to 1992) Inclusive

The Nova Scotian Institute of Science has published facts about the natural history of the Province (and also of much of Canada) in its Proceedings since 1863. The Proceedings are therefore important, not only to students of the history of science, but to all those scientists, now and in the future, whose interests require a knowledge of their predecessors work. The Proceedings are difficult to access, because volumes 1-8 are hard to find and in addition no index has been published since volume 26. A cumulative index to volumes 1-7 was published (Proc. N.S. Inst. Sci. 7: 495-523) in 1890 and a further cumulative index to volumes 1-25 in 1953. Neither of these segregated a list of authors from a subject index and the latter covered information found only in the titles of papers and abstracts.

Bibliographic and editorial work for the Institute has become increasingly difficult in recent years because of the problem of finding information published in the Proceedings. The Council of the Institute therefore authorized a thorough cumulative index of Volumes 1-39 inclusive and this is now published as separate author and subject indices. Certain conventions have been adopted in the assembly of these indices and these are described in the next two sections of this introduction.

Author index

All authors (825) of papers and abstracts are included in this index and are given in strict alphabetical order (e.g. MacA... appears before McA...). The titles of some papers are slightly expanded to give a better indication of their content. In such cases additional material is enclosed in parentheses, care must therefore be taken to distinguish editorial additions from e.g., parenthetic binominal addenda. In other cases, titles have been abbreviated slightly to standardize the format of the index. Throughout, where authors have used terms that have been superseded by internationally agreed nomenclature, the terms have been replaced. As far as possible units have been converted into the centimetre-second-gram system. Prefixes used are translated in the table of abbreviations found at the end of the subject index. Abstracts are distinguished from full papers by an asterisk. The titles of all abstracts are given, even those that contain minimal information. A full list of references is given for each author, but the titles of papers and abstracts having more than one author are only given for the author first named.

Subject Index

About 80% of the papers published in the Proceedings could be classified as biology. The subject index has therefore been built by assembling a list of the binominal names of plants and animals that form the subject of original botanical and zoological studies. A consistent use of authorities has not been achieved for many reasons and this is an area that might well be improved in future editions of this index. Species that are merely mentioned in catalogues (e.g. floras) are not included, and species reported in reviews are only included if they are judged to illustrate, or alert the reader to the subject matter. Obviously this judgement is biased, but can be corrected in future editions. The index incorporates a glossary of common English names of species and their corresponding scientific nomenclature. This has been done to enable the use of binominal names throughout the index because the Proceedings are exchanged with many scientific societies whose members might be unfamiliar with these common names. There are, of course many instances where the taxonomy of species has been changed and an attempt has been made to give cross references in these cases.

There are, however, many papers that describe work of general biological interest particularly in the fields of physiology and biochemistry. Thus in addition to the list of organisms there is a section of the index devoted to biology that is subdivided into the various sub-disciplines, with the exceptions of agriculture, horticulture, phenology and paleontology. The first two of these subjects are combined in a separate section and the latter finds its traditional place as a subdiscipline of geology. In cross referencing the main subject heading e.g. "biology" is given first followed by the subdiscipline or subject e.g. "ecology". The phenological data, collected over 31 years by Dr. A.H. MacKay, are a major contribution to Canadian science and are given in a separate section.

An attempt has been made to provide a geographical index to allow those who for example, are interested in the ecology of an area to be easily able to find all references to that location. In Nova Scotia it has been somewhat inconsistently divided into papers dealing generally with the Province, and papers classified on a county basis with one exception. This deals with Cape Breton. Elsewhere, subdivision is made only to provincial or state level.

Most papers on other scientific disciplines can be found under the appropriate heading, except for geology and physics which are divided into the usual sub-disciplines. There are very few papers dealing with purely chemical investigations. Thus chemical aspects of papers are classified under three headings: analytical methods and techniques, minerals, and chemical substances. Papers on physical chemical topics, especially thermodynamics, are given under the appropriate sub-heading in physics.

The chemical substances section is arranged, more or less, in accord with Chemical Abstracts conventions. All substances are given in alphabetical order. Carbon compounds are given (where possible) in order of increasing numbers of carbon atoms in the molecule and within each set of compounds having the same numbers of carbon atoms, in increasing numbers of *hydrogen* atoms i.e. not in alphabetical order. Within each group of carbon and hydrogen atoms the remaining elements in the molecules are given in alphabetical order. As in Chemical Abstracts usage the technique of roots is occasionally used. Thus derivatives of 2-amino-3-phenylpropan-1-ol are given under this heading in order of increasing molecular weight. Here and throughout the subject index a dash "-" is used to indicate repetition of words on the previous line. Selection rules for chemicals to appear in this subsection of the index were similar to the selection of species (see above), particularly in the case of review papers. Throughout the indices, symbols for chemical elements are used that are recommended by the International Union of Pure and Applied Chemistry. In formulae numerical subscripts indicate multiple atomic species in the molecule, numerical superscripts preceding the symbol indicate abnormal isotopic species, and those following the symbol(s) ionic charge.

Many subjects reported in abstracts give little or no factual data; these are not incorporated into the subject index unless a reference is given in the abstract to full publication of the experimental data in another journal. Where space is available this journal name is indicated in the index. Several instruments and other measuring equipment are mentioned in abstracts and papers but are not included in the subject index unless sufficient details e.g. drawings, are given to allow them to be constructed or bought.

The assembly of these indices has been facilitated by the use of a computer and as a result the texts are available in Macintosh or IBM format on 800 Kbyte or 1.44 Mbyte 3.5" discs or on 5.25" floppy discs. Copies of these discs can be purchased from the Institute for $25.00. When ordering the disc please state the format and type of disc required. The information on the discs is in text format and can be read in any of the usual word-processing utilities. Alternatively the discs can be supplied in one (e.g. Wordperfect) of these formats.

Many scientists will find these discs more useful than the printed form because they can be expanded by their owners to incorporate data published in future and/or additional data on topics that are of particular interest to them. The Editors hope that users of the index will report the errors and omissions that they find, so that the electronic version of the index, at least, can be continually improved.

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Ambrose, J.		10: IV; 13: XC.
Belt, T.	geologist	13: XCII.
Bowman, M.	public analyst	15: XVIII.
Bronson, H.L.,	president 1918; physicist	26: 272-273.
Brown, M.	bryologist	25: 272.
Brown, R.	mining engineer	13: LVII
Coffin, C.C.,	president 1948; chemist	24: 41.
Dawson, W.	geologist	10: XXXVI.
DeWolfe, J.R.,		10: LXXXIV.
Downs, A.	zoologist	8(3): XLVIII.
-	founder: first zoological garden in America	10: CII; 11: XI
Edelstein, T.	botanist	28: 121-122.
Ells, R.W.	geologist	13: XXV.
Fergusson, D. MacE.,	president 1912, chemist	16: XVIII.
Fletcher, H.		12: LVIII.
Forbes, J.,	mechanical engineer	14: XII.
Fox, J.J.		10: XXXVI; 13: CVII.
Gesner, A.,	mineralogist	13: LV.
Gilpin, E.,	president 1895; mining engineer	12: XXXI.
Gilpin, J.B.,	president 1873; zoologist	8(3): XLVII.
Gossip, W.,	president 1878; anthropologist	7: 319-320.
Guptill, E.W.	physicist	27: 180.
Haliburton, R.G.,	anthropologist	13: XC.
Hardy, C.	soldier & naturalist	15: VII.
Harvey, M.,		10: LXXXV.
Haycock, E.	geologist	15: XVII.
Hess, E.	president 1942; editor 1940-1949 (not obit.)	22: III.
Hill, P.C.	president 1862;	13: LXXXI.
Honeyman, D.;	geologist	7: 313; 320.
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How, H.	chemist & mineralogist	13: XCIV.
Johnstone, J.H.L.	president 1926; physicist	27: 102.
Jones, J.M.	president 1863-1873	10: LXXX.
Lawson, G.,	president 1893; botanist	9: XXIII.
Longard, J.R.	physicist	28: 123-124.
Kennedy, G.T.,	chemist	12: XXXV.
MacCulloch, T.,	mineralogist	13: LV.
MacGregor, J.G.	president 1888, chemist	14: LXXV.
MacKay, A.H.	president 1899; editor 1908-1929	17: XLVII.
Marcou, J.	geologist	10: IV.
Masson, C.R.	president, 1964; treasurer, 1986; chemist	38: 189-198.

McKay, A.	president, 1897	14: LXVIII.
McKay, E.	president, 1907	15: XXVII.
McKerron, W.	treasurer 1903; agriculturalist	14: XII.
Morrow, R.	president 1883	13: LXXXV.
Myers, W.J.	soldier	13: XCI.
Neish, A.C.	president 1969; botanist	27: 103; 104-6.
Nickerson, C.B.	president 1922; chemist	20: 289.
Parker, D.McN,	surgeon	12: XXXIV.
Patterson, G.	archaeologist & historian	9: XCV.
Poole, H.S.	president 1902	14: LXVIII.
Silver, W.C.	treasurer 1867-1903	13: CI.
Smith, T.	(1768-1850)	1(4): 149; 13: LIII.
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Somers, J.	president 1885; physiologist & botanist	10: III.
Stewart, D.K.R.	chemist	28: 125.
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Webster, W.B.	mineralogist	13: LVI.
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Yaphe, W.	secretary 1960; bacteriologist	37: 149-152.
Young, E.G.	president 1930; editor 1964-71; biochemist	27: 182-184.
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ABBREVIATIONS USED IN THE INDICES

An attempt has been made to avoid abbreviations (including their use by authors) but inevitably some have been used to maintain a consistant format. The following is an incomplete list whose omissions are largely confined to authorities for binominal names.

Arg	arginine; 2-amino-5-guanidinovaleric acid
Assoc.	association
B.C.	British Columbia
Btu	Brtish thermal unit (1 Btu = 1,054.8 joules)
Can.	Canada
cm	centimetre (metre x 10^{-2})
Co.	county
E	east
Et	ethyl
etc.	including other inferred or related matters
fam.	family (in a taxonomic sense)
fg	femtogram (gram x 10^{-15})
Fig.	figure (illustration)
Fr.	Fries
ft.	foot (1 foot = 0.3048 metres)
g	gram
Geol.	geology
Glu	glucose
h	hour
Herb	herbarium (usually including accession number)
His	histidine; 2-amino-3-(imidazolyl-5')propionic acid
Hoffm.	Hoffmann

Hz	hertz (cycles per second)
incl.	including
J.	journal
k	kilo (x 10^3)
L.	Linneas
Lamb.	Lambert
Leu	leucine; 2-amino-4-methylvaleric acid
Lib.	Libert
M	mega (x 10^6)
m	metre
M.Sc.	Master of Science degree
Man.	Manitoba
max.	maximum
Me	methyl
mg	milligram (10^{-3} gram)
MHz	megahertz (hertz x 10^6)
mL	millilitre (Litre x 10^{-3})
Mm	megametres (metre x 10^6)
N	north
N.B.	New Brunswick
N.S.	Nova Scotia
nov. sp. (n. sp.)	plant or animal described for the first time
°C	degrees Celsius
Ont.	Ontario
P.E.I.	Prince Edward Island
pg	picogram (gram x 10^{-12})
Phe	phenylalanine; 2-amino-3-phenylpropionic acid
Proc.	proceedings
Pt.	point (usually geographical)
Pub.	public
Qué	Québec
R.	river
Raf	raffinose
S	south
s	second
Sask.	Saskatchewan
sp.	species
spp.	species (plural)
ssp.	sub-species
St.	Saint (usually in geographical name)
Stack.	Stackhouse
Suc	sucrose
U	university
U.K.	United Kingdom of Great Britain & Northern Ireland
U.S.A.	United States of America
vit.	vitamin
W	west
Xyl	xylose
y	year

PRESIDENT'S REPORT 1991-92

During the past year, the Institute has enjoyed a wide ranging series of lectures held at the Nova Scotia Museum. The topics for the year were as follows:

October: Schizophrenia, Epilepsy, and Changes in Neuronal Gene Expression
Professor Harold Robertson, Dept. of Pharmacology,
Dalhousie University
November: Awareness of Industrial Ergonomics in Nova Scotia
Professor Biman Das., Technical University of Nova Scotia
December: Singing on a Light-Beam: Trans-Atlantic Fibre Optic
Telecommunication
Professor Barry Paton, Dept. of Physics, Dalhousie University
January: Properties of Matter: From Fudge to Photocopying
Professor Mary Anne White, Dept. of Chemistry,
Dalhousie University
February: Three Kingdoms: The Earliest Stages in Cellular Evolution
Professor W. Ford Doolittle, Dept. of Biochemistry,
Dalhousie University
March: The "Health" of Our Ocean
Dr. R.F. Addison, Bedford Institute of Oceanography
April: The Prince Edward Island "Fixed" Link
Director, Northumberland Strait Crossing Project

The attendance at the meetings was good, averaging somewhat over thirty per meeting.

Another important means of communication for the Institute is the written word. Your Editor's Report has reviewed the publication of the Proceedings of the Institute, and the Librarian has reported concerning our collection which is held at the Science Library at Dalhousie University. The Staff there are very protective of our collection, and I would like to acknowledge their support.

The Institute has entered into negotiations with NRC with regard to acquiring and circulating, on a cost recovery basis, their computer data base on the availability of fungal isolates and cultures in Canada.

Over the course of the past year the Council of the Institute has discussed our membership numbers on several occasions. In that regard, we have laid the foundation for a membership drive during the forthcoming year. The recommendations of the current Council will be presented to you as the next item of business on the agenda of this Annual Meeting. In addition, the Institute has maintained close contact with the Science Teachers of Nova Scotia and expect to add one of their members to the Institute's Council.

During the Province-wide Science and Technology Week in October of this year, the Institute will be sponsoring a special public lecture in addition to our regular meeting. This will be held immediately prior to the commencement of the Nova Scotia Science Teachers Inservice gathering so as to be readily accessible to them as well as the general public.

I would like to acknowledge the support of the Nova Scotia Museum who continue to proved us with financial support for the Proceedings and to provide meeting space for us.

Finally, I would like to thank the members of the Institute's Council who have consistently given of their time and energy in supporting the Institute during the year. As a result of their cooperation, I have had an enjoyable period as President.

David Goble

NOVA SCOTIAN INSTITUTE OF SCIENCE
BALANCE SHEET
AS OF MARCH 31, 1991

ASSETS:

Current Account	($17.63)		
Savings Account	$2,390.47		
Wood Gundy Account	$258.86		
Petty Cash	$42.59		
TOTAL CASH		$2,674.29	
INVESTMENTS		$31,736.19	$34,410.48
ACCOUNTS RECEIVALBE			$1,304.56
TOTAL ASSETS			$35,715.04

LIABILITIES AND NET WORTH:

ACCOUNTS PAYABLE		$3,178.85
DEFERRED REVENUE		$30.60
PERMANENT FUND	$3,550.14	
SURPLUS AS OF APRIL 1/89	$27,942.31	
EXCESS INCOME/(EXPENSES)	$1,013.14	$32,505.59
TOTAL LIABILITIES AND NET WORTH		$35,715.04

INVESTMENTS:

Maturing Dec. 9/92 @ 10.5%	$1,000.00	
Maturing April 12/91 @ 8.95%	$30,736.19	
		$31,736.19

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INSTRUCTIONS TO AUTHORS

The *Proceedings of the Nova Scotian Institute of Science* publishes the proceedings of the Institute and original articles, including notes, pertaining to the natural sciences of the Atlantic Provinces, especially in biology and geology. Manuscripts may be either in English or French, and acceptance for publication is based on recommendations of referees.
Manuscripts should be typewritten, double-spaced on white paper 21.5 by 28 cm (8.5 by 11 in) with margins of 4 cm (1.5 in). Underline only material to be set in italics, and use capital letters only when letter or words should appear in capitals in the printed paper. Layout and style should follow that used in this volume. The original copy and one duplicate are required. Each page of the manuscript should be numbered, the first page carrying only the title, authors' names and affiliations, and any necessary footnotes.

Spelling should follow that of **Webster's Third New International Dictionary**, and authors are responsible for consistency in spelling.

Abbreviations, nomenclature, and symbols for units of measurements should follow international recommendations. Metric units, SI units and decimals should be used whenever possible. Use day/month/year sequence for dates. Do not use periods after such abbreviations as "mm, kg, DOE, NRC, etc."

Taxonomic keys should be in aligned-couplet form in zoology and paleontology, and in multi-level indent form in botany. Synonomy in botany, zoology, and paleontology should be in the short form—taxon, author, year, page—with full citation in the references.

An abstract of not more than 200 words should precede the body of the text. This should be followed by an introduction, methods, results or observations, discussion, and references. In some cases a combination of these sections may be more effective.

References should be checked with the original article and referred to in the text by author and date, in parentheses. References should be listed alphabetically at the end of the manuscript. Reference to papers in periodicals must include author, date, title, journal, volume number, and inclusive pagination. Serials are abbreviated in the form given in **Bibliographic Guide for Editors and Authors** (the American Chemical Society, 1974). For serials not found therein, the abbreviated name in the **World List of Scientific Periodicals** (Butterworths, 1963) is used. The following are examples of style for references from journals, books, and symposium volumes:

Author, A.N. and Other, T.H.E. 1978. Title of the article. *Name of Journal,* (Volume) 99: 1-10. (Omit word volume)
Author, A.N. and Coauthor, A. 1978. *Full Title of Book*. Publisher, City.
Author, A. 1978. Title of Article. In *Title of Book or Symposium Volume*. (ed. J. Smith and B. Jones). Publisher, City, pp. 256-301.

Tables should be numbered with roman numerals, having a brief title, and referred to in the text. Vertical rules should not be used. Tables must be typed separately from the text on individual pages.

Originals of illustrations should not be more than three times the size of the final reproduction, 11 by 18 cm. Figures, including those in plates, are numbered consecutively in arabic numerals, and each referred to in the text. Original drawings, and one set of clean copy, are required and should be made with india ink on white paper. All lines must be sufficiently thick to reproduce well, and letters and characters sufficiently large to be legible and not less than 1 mm high when reduced. Captions for illustrations should be typed separately from the text.

Photographs should be mounted on heavy bristol board, and ready for reproduction. Prints must be of high quality on glossy paper with no space between those mounted in groups. Photographs of poor quality will be rejected and this, in turn, may require extensive revamping of the ms.

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